de la Propriété
le
du Canada
Un organisme
d'Industrie Canada

EXPRESS MAIL NO.
EV889128890US

An agency of
Industry Canada

CA 2438921 A1 2002/08/29

(21) 2 438 921

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2002/02/21
(87) Date publication PCT/PCT Publication Date: 2002/08/29
(85) Entrée phase nationale/National Entry: 2003/08/20
(86) N° demande PCT/PCT Application No.: CA 2002/000207
(87) N° publication PCT/PCT Publication No.: 2002/066650
(30) Priorité/Priority: 2001/02/21 (60/269,840) US

(51) Cl.Int.^{7}/Int.Cl.^{7} C12N 15/31, A61K 39/09, A61P 31/00, A61P 17/00, C07K 14/315, C12N 15/63, C07K 19/00, G01N 33/569

(71) Demandeur/Applicant:
SHIRE BIOCHEM INC., CA

(72) Inventeurs/Inventors:
MARTIN, DENIS, CA;
RIOUX, STEPHANE, CA;
BRODEUR, BERNARD R., CA;
HAMEL, JOSEE, CA;
RHEAULT, PATRICK, CA

(74) Agent: SMART & BIGGAR

(54) Titre : POLYPEPTIDES DE STREPTOCOCCUS PYOGENES ET FRAGMENTS D'ADN CORRESPONDANTS
(54) Title: STREPTOCOCCUS PYOGENES POLYPEPTIDES AND CORRESPONDING DNA FRAGMENTS

```
   1 MKKHLKTVAL TLTTVSVVTH NQEVFSLVKE PILKQTQASS SISGADYAES SGKSKLKINE
  61 TSGPVDDTVT DLFSDKRTTP EKIKDNLAKG PREQELKAVT ENTESEKQIT SGSQLEQSKE
 121 SLSLNKTVPS TSNWEICDFI TKGNTLVGLS KSGVEKLSQT DHLVLPSQAA DGTQLIQVAS
 181 FAFTPDKKTA IAEYTSRAGE NGEISQLDVD GKEIINEGEV FNSYLLKKVT IPTGYKHIGQ
 241 DAFVDNKNIA EVNLPESLET ISDYAFAHLA LKQIDLPDNL KAIGELAFFD NQITGKLSLP
 301 RQLMRLAERA FKSNHIKTIE FRGNSLKVIG EASFQDNDLS QLMLPDGLEK IESEAFTGNP
 361 GDDHYNNRVV LWTKSGKNPS GLATENTYVN PDKSLWQESP EIDYTKWLEE DFTYQKNSVT
 421 GFSNKGLQKV KRNKNLEIPK QHNGVTITEI GDNAFRNVDF QNKTLRKYDL EEVKLPSTIR
 481 KIGAFAFQSN NLKSFEASDD LEEIKEGAFM NNRIETLELK DKLVTIGDAA FHINHIYAIV
 541 LPESVQEIGR SAFRQNGANN LIFMGSKVKT LGEMAFLSNR LEHLDLSEQK QLTEIPVQAF
 601 SDNALKEVLL PASLKTIREE AFKKNHLKQL EVASALSHIA FNALDDNDGD EQFDNKVVVK
 661 THHNSYALAD GEHFIVDPDK LSSTIVDLEK ILKLIEGLDY STLRQTTQTQ FRDMTTAGKA
 721 LLSKSNLRQG EKQKFLQEAQ FFLGRVDLDK AIAKAEKALV TKKATKNGQL LERSINKAVL
 781 AYNNSAIKKA NVKRLEKELD LLTGLVEGKG PLAQATMVQG VYLLKTPLPL PEYYIGLNVY
 841 FDKSGKLIYA LDMSDTIGEG QKDAYGNPIL NVDEDNEGYH ALAVATLADY EGLDIKTILN
 901 SKLSQLTSIR QVPTAAYHRA GIFQAIQNAA AEAEQLLPKP GTHSEKSSSS ESANSKDRGL
 961 QSNPKTNRGR HSAILPRTGS KGSFVYGILG YTSVALLSLI TAIKKKKY*
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(57) Abrégé/Abstract:
The present invention relates to antigens, more particularly antigens of Streptococcus pyogenes (also called group A Streptococcus (GAS)) bacterial pathogen which are useful as vaccine component for prophylaxis, therapy and/or diagnostic.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 August 2002 (29.08.2002)

PCT

(10) International Publication Number
WO 02/066650 A3

(51) International Patent Classification[7]: C12N 15/31, 15/63, C07K 14/315, 19/00, A61K 39/09, A61P 17/00, 31/00, G01N 33/569

(21) International Application Number: PCT/CA02/00207

(22) International Filing Date: 21 February 2002 (21.02.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/269,840 21 February 2001 (21.02.2001) US

(71) Applicant *(for all designated States except US)*: **SHIRE BIOCHEM INC.** [CA/CA]; 275 Armand Frappier Boulevard, Laval, Quebec H7V 4A7 (CA).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **MARTIN, Denis** [CA/CA]; 4728-G, rue Gaboury, St-Augustin-de-Desmaures, Quebec G3A 1E9 (CA). **RIOUX, Stéphane** [CA/CA]; 869 Avenue Des Pinsons, Beauport, Québec G1E 1J3 (CA). **BRODEUR, Bernard, R.** [CA/CA]; 2401 Maritain, Sillery, Quebec G1T 1N6 (CA). **HAMEL, Josée** [CA/CA]; 2401 Maritain, Sillery, Quebec G1T 1N6 (CA). **RHEAULT, Patrick** [CA/CA]; 44 rue Bélair, St-Etienne-de-Lauzon, Québec G6J 1P9 (CA).

(74) Agents: **MORROW, Joy, D.** et al.; Smart & Biggar, P.O. Box 2999, Station D, 900-55 Metcalfe Street, Ottawa, Ontario K1P 5Y6 (CA).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

[Continued on next page]

(54) Title: STREPTOCOCCUS PYOGENES POLYPEPTIDES AND CORRESPONDING DNA FRAGMENTS

(SEQ ID NO:2)

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  1 MKKHLKTVAL TLTTVSVVTH NQEVFSLVKE PILKQTQASS SISGADYAES SGKSKLKINE
 61 TSGPVDDTVT DLFSDKRTTP EKIKDNLAKG PREQELKAVT ENTESEKQIT SGSQLEQSKE
121 SLSLNKTVPS TSNWEICDFI TKGNTLVGLS KSGVEKLSQT DHLVLPSQAA DGTQLIQVAS
181 FAFTPDKKTA IAEYTSRAGE NGEISQLDVD GKEIINEGEV FNSYLLKKVT IPTGYKHIGQ
241 DAFVDNKNIA EVNLPESLET ISDYAFAHLA LKQIDLPDNL KAIGELAFFD NQITGKLSLP
301 RQLMRLAERA FKSNHIKTIE FRGNSLKVIG EASFQDNDLS QLMLPDGLEK IESEAFTGNP
361 GDDHYNNRVV LWTKSGKNPS GLATENTYVN PDKSLWQESP EIDYTKWLEE DFTYQKNSVT
421 GFSNKGLQKV KRNKNLEIPK QHNGVTITEI GDNAFRNVDF QNKTLRKYDL EEVKLPSTIR
481 KIGAFAFQSN NLKSFEASDD LEEIKEGAFM NNRIETLELK DKLVTIGDAA FHINHIYAIV
541 LPESVQEIGR SAFRQNGANN LIFMGSKVKT LGEMAFLSNR LEHLDLSEQK QLTEIPVQAF
601 SDNALKEVLL PASLKTIREE AFKKNHLKQL EVASALSHIA FNALDDNDGD EQFDNKVVVK
661 THHNSYALAD GEHFIVDPDK LSSTIVDLEK ILKLIEGLDY STLRQTTQTQ FRDMTTAGKA
721 LLSKSNLRQG EKQKFLQEAQ FFLGRVDLDK AIAKAEKALV TKKATKNGQL LERSINKAVL
781 AYNNSAIKKA NVKRLEKELD LLTGLVEGKG PLAQATMVQG VYLLKTPLPL PEYYIGLNVY
841 FDKSGKLIYA LDMSDTIGEG QKDAYGNPIL NVDEDNEGYH ALAVATLADY EGLDIKTILN
901 SKLSQLTSIR QVPTAAYHRA GIFQAIQNAA AEAEQLLPKP GTHSEKSSSS ESANSKDRGL
961 QSNPKTNRGR HSAILPRTGS KGSFVYGILG YTSVALLSLI TAIKKKKY*
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(57) Abstract: The present invention relates to antigens, more particularly antigens of Streptococcus pyogenes (also called group A Streptococcus (GAS)) bacterial pathogen which are useful as vaccine component for prophylaxis, therapy and/or diagnostic.

WO 02/066650 A3

WO 02/066650 A3

(88) Date of publication of the international search report:
31 October 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

STREPTOCOCCUS PYOGENES POLYPEPTIDES AND CORRESPONDING DNA FRAGMENTS

FIELD OF THE INVENTION

The present invention is related to polypeptides of Streptococcus pyogenes (Group A Streptococcus) which may be used to prevent, diagnose and/or treat streptococcal infection.

BACKGROUND OF THE INVENTION

Streptococci are gram (+) bacteria which are differentiated by group specific carbohydrate antigens A through O which are found at the cell surface. S. pyogenes isolates are further distinguished by type-specific M protein antigens. M proteins are important virulence factors which are highly variable both in molecular weights and in sequences. Indeed, more than 80-M protein types have been identified on the basis of antigenic differences.

S. pyogenes is responsible for many diverse infection types, including pharyngitis, erysipelas and impétigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis. A resurgence of invasive disease in recent years has been documented in many countries, including those in North America and Europe. Although the organism is sensitive to antibiotics, the high attack rate and rapid onset of sepsis results in high morbidity and mortality.

To develop a vaccine that will protect hosts from S. pyogenes infection, efforts have focused on virulence factors such as the type-specific M proteins. However, the amino-terminal portion of M proteins was found to induce cross-reactive antibodies which reacted with human myocardium, tropomyosin, myosin, and vimentin, which might be implicated in autoimmune diseases. Others have used recombinant techniques to produce complex hybrid proteins containing amino-terminal peptides of M proteins from different serotypes. However, a safe vaccine containing all

S. pyogenes serotypes will be highly complex to produce and standardize.

In addition to the serotype-specific antigens, other S. pyogenes proteins have generated interest as potential vaccine candidates. The C5a peptidase, which is expressed by at least S. pyogenes 40 serotypes, was shown to be immunogenic in mice, but its capacity to reduce the level of nasopharyngeal colonization was limited. Other investigators have also focused on the streptococcal pyrogenic exotoxins which appear to play an important role in pathogenesis of infection. Immunization with these proteins prevented the deadly symptoms of toxic shock, but did not prevent colonization.

The University of Oklahoma has set up a genome sequencing project for S. pyogenes strain M1 GAS (http://dna1.chem.ou.edu/strep.html).

Therefore there remains an unmet need for S. pyogenes antigens that may be used vaccine components for the prophylaxis and/or therapy of S. pyogenes infection.

SUMMARY OF THE INVENTION

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID No : 2 or fragments or analogs thereof.

According to one aspect, the present invention relates to polypeptides which comprise an amino acid sequence SEQ ID No : 2 or fragments or analogs thereof.

In other aspects, there are provided polypeptides encoded by polynucleotides of the invention, pharmaceutical compositions, vectors comprising polynucleotides of the invention operably linked to an expression control region, as well as host cells transfected with said vectors and processes for producing polypeptides comprising culturing said host cells under conditions suitable for expression.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 represents the DNA sequence of BVH-P7 gene from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 1. The underlined portion of the sequence represents the region coding for the leader peptide.

Figure 2 represents the amino acid sequence BVH-P7 protein from serotype M1 S. pyogenes strain ATCC700294; SEQ ID NO: 2. The underline sequence represents the 21 amino acid residues leader peptide.

Figure 3 depicts the comparison of the predicted amino acid sequences of the BVH-P7 open reading frames from Spy74 (SEQ ID NO: 3), Spy70 (SEQ ID NO: 4), Spy69 (SEQ ID NO: 5), Spy68 (SEQ ID NO: 6), Spy 60 (SEQ ID NO: 7), ATCC12357 (SEQ ID NO: 8), ATCC700294 (SEQ ID NO: 2), S. pyogenes strains by using the program Clustal W from MacVector sequence analysis software (version 6.5). Underneath the alignment, there is a consensus line where * and . characters indicate identical and similar amino acid residues, respectively.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides purified and isolated polynucleotides, which encode Streptococcal polypeptides that may be used to diagnose, prevent, and/or treat Streptococcal infection.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect of the present invention, there is provided an isolated polypeptide comprising a polypeptide chosen from: (a) a polypeptide comprising SEQ ID NO: 2; (b) a polypeptide comprising an antigenic or immunogenic fragment having at least 10 contiguous amino acid residues of the polypeptide of (a); (c) a polypeptide comprising an antigenic or immunogenic analog having at least 70% identity to the polypeptide of (a) or (b); (d) a polypeptide comprising an antigenic or immunogenic analog having at least 95% identity to the polypeptide of (a) or (b); (e) a polypeptide capable of generating antibodies having binding specificity for the polypeptide of any one of (a), (b), (c) and (d); (f) an epitope bearing portion of the polypeptide of any one of (a), (b), (c) and (d); (g) the polypeptide of any one of (a), (b), (c), (d), (e) and (f) wherein the N-terminal Met residue is deleted; and (h) the polypeptide of any one of (a), (b), (c), (d), (e), (f) and (g) wherein the secretory amino acid sequence is deleted.

According to another aspect of the present invention, there is provided an isolated polypeptide comprising a polypeptide chosen from: (a) a polypeptide comprising SEQ ID NO: 2; (b) a polypeptide having at least 70% identity to the polypeptide of (a); (c) a polypeptide having at least 95% identity to the polypeptide of (a); (d) a polypeptide capable of generating antibodies having binding specificity for the polypeptide of (a); (e) an epitope bearing portion of the polypeptide of (a); (f) the

polypeptide of any one of (a), (b), (c), (d) and (e) wherein the N-terminal Met residue is deleted; and (g) the polypeptide of any one of (a), (b), (c), (d), (e) and (f) wherein the secretory amino acid sequence is deleted.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least

90% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides a polynucleotide encoding an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide comprising SEQ ID NO: 2 or fragments or analogs or thereof.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 80% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 90% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention relates to polypeptides characterized by the amino acid sequence comprising SEQ ID NO: 2.

According to one aspect, the present invention provides a polynucleotide encoding an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention relates to epitope bearing portions of a polypeptide comprising SEQ ID NO: 2.

According to one aspect, the present invention provides an isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(b) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(c) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(d) a polynucleotide encoding a polypeptide capable of generating antibodies having binding specificity for a polypeptide comprising a sequence chosen from: SEQ ID NO: 2 or fragments or analogs thereof;

(e) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NO: 2 or fragments or analogs thereof;

(f) a polynucleotide comprising a sequence chosen from SEQ ID NO: 1 or fragments or analogs thereof;

(g) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e) or (f).

According to one aspect, the present invention provides an isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide encoding a polypeptide having at least 70% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(b) a polynucleotide encoding a polypeptide having at least 95% identity to a second polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(c) a polynucleotide encoding a polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(d) a polynucleotide encoding a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising a sequence chosen from: SEQ ID NO: 2;

(e) a polynucleotide encoding an epitope bearing portion of a polypeptide comprising a sequence chosen from SEQ ID NO: 2;

(f) a polynucleotide comprising a sequence chosen from SEQ ID NO: 1;

(g) a polynucleotide that is complementary to a polynucleotide in (a), (b), (c), (d), (e) or (f).

According to one aspect, the present invention provides an isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(b) a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(c) a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(d) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(e) an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof;

(f) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the secretory amino acid sequence is deleted.

According to one aspect, the present invention provides an isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide having at least 70% identity to a second polypeptide comprising SEQ ID NO: 2;

(b) a polypeptide having at least 95% identity to a second polypeptide comprising SEQ ID NO: 2;

(c) a polypeptide comprising SEQ ID NO: 2;

(d) a polypeptide capable of raising antibodies having binding specificity for a polypeptide comprising SEQ ID NO: 2;

(e) an epitope bearing portion of a polypeptide comprising SEQ ID NO: 2;

(f) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the N-terminal Met residue is deleted;

(g) the polypeptide of (a), (b), (c), (d), (e) or (f) wherein the secretory amino acid sequence is deleted.

Those skilled in the art will appreciate that the invention includes DNA molecules, i.e. polynucleotides and their complementary sequences that encode analogs such as mutants, variants, homologues and derivatives of such polypeptides, as described herein in the present patent application. The invention also includes RNA molecules corresponding to the DNA molecules of the invention. In addition to the DNA and RNA molecules, the invention includes the corresponding polypeptides and monospecific antibodies that specifically bind to such polypeptides.

In a further embodiment, the polypeptides in accordance with the present invention are antigenic.

In a further embodiment, the polypeptides in accordance with the present invention are immunogenic.

In a further embodiment, the polypeptides in accordance with the present invention can elicit an immune response in a host.

In a further embodiment, the present invention also relates to polypeptides which are able to raise antibodies having binding specificity to the polypeptides of the present invention as defined above.

An antibody that "has binding specificity" is an antibody that recognizes and binds the selected polypeptide but which does not substantially recognize and bind other molecules in a sample, e.g., a biological sample. Specific binding can be measured using an ELISA assay in which the selected polypeptide is used as an antigen.

In accordance with the present invention, "protection" in the biological studies is defined by a significant increase in the survival curve, rate or period. Statistical analysis using the Log rank test to compare survival curves, and Fisher exact test to compare survival rates and numbers of days to death, respectively, might be useful to calculate P values and determine whether the difference between the two groups is statistically significant. P values of 0.05 are regarded as not significant.

In an additional aspect of the invention there are provided antigenic/immunogenic fragments of the polypeptides of the invention, or of analogs thereof.

The fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties. Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a polypeptide or analog thereof as described herein. The present invention further provides fragments having at least 10 contiguous amino acid residues from the polypeptide sequences of the present invention. In one embodiment, at least 15 contiguous amino acid residues. In one embodiment, at least 20 contiguous amino acid residues.

The key issue, once again, is that the fragment retains the antigenic/immunogenic properties.

The skilled person will appreciate that analogs of the polypeptides of the invention will also find use in the context of the present invention, i.e. as antigenic/immunogenic material. Thus, for instance proteins or polypeptides which include one or more additions, deletions, substitutions or the like are encompassed by the present invention.

As used herein, "fragments", "analogs" or "derivatives" of the polypeptides of the invention include those polypeptides in which one or more of the amino acid residues are substituted

with a conserved or non-conserved amino acid residue (preferably conserved) and which may be natural or unnatural. In one embodiment, derivatives and analogs of polypeptides of the invention will have about 70% identity with those sequences illustrated in the figures or fragments thereof. That is, 70% of the residues are the same. In a further embodiment, polypeptides will have greater than 80% identity. In a further embodiment, polypeptides will have greater than 85% identity. In a further embodiment, polypeptides will have greater than 90% identity. In a further embodiment, polypeptides will have greater than 95% identity. In a further embodiment, polypeptides will have greater than 99% identity. In a further embodiment, analogs of polypeptides of the invention will have fewer than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10.

These substitutions are those having a minimal influence on the secondary structure and hydropathic nature of the polypeptide. Preferred substitutions are those known in the art as conserved; i.e. the substituted residues share physical or chemical properties such as hydrophobicity, size, charge or functional groups. These include substitutions such as those described by Dayhoff, M. in Atlas of Protein Sequence and Structure 5, 1978 and by Argos, P. in EMBO J. 8, 779-785, 1989. For example, amino acids, either natural or unnatural, belonging to one of the following groups represent conservative changes:
ala, pro, gly, gln, asn, ser, thr, val;
cys, ser, tyr, thr;
val, ile, leu, met, ala, phe;
lys, arg, orn, his;
and phe, tyr, trp, his.
The preferred substitutions also include substitutions of D-enantiomers for the corresponding L-amino acids.

In an alternative approach, the analogs of the polypeptides of the invention comprise the substitutions disclosed in Figure 3.

In an alternative approach, the analogs could be fusion proteins, incorporating moieties which render purification easier, for example by effectively tagging the desired

polypeptide. It may be necessary to remove the "tag" or it may be the case that the fusion polypeptide itself retains sufficient antigenicity to be useful.

The percentage of homology is defined as the sum of the percentage of identity plus the percentage of similarity or conservation of amino acid type.

In one embodiment, analogs of polypeptides of the invention will have about 70% identity with those sequences illustrated in the figures or fragments thereof. That is, 70% of the residues are the same. In a further embodiment, polypeptides will have greater than 75% homology. In a further embodiment, polypeptides will have greater than 80% homology. In a further embodiment, polypeptides will have greater than 85% homology. In a further embodiment, polypeptides will have greater than 90% homology. In a further embodiment, polypeptides will have greater than 95% homology. In a further embodiment, polypeptides will have greater than 99% homology. In a further embodiment, analogs of polypeptides of the invention will have fewer than about 20 amino acid residue substitutions, modifications or deletions and more preferably less than 10.

One can use a program such as the CLUSTAL program to compare amino acid sequences. This program compares amino acid sequences and finds the optimal alignment by inserting spaces in either sequence as appropriate. It is possible to calculate amino acid identity or similarity (identity plus conservation of amino acid type) for an optimal alignment. A program like BLASTx will align the longest stretch of similar sequences and assign a value to the fit. It is thus possible to obtain a comparison where several regions of similarity are found, each having a different score. Both types of identity analysis are contemplated in the present invention.

In an alternative approach, the analogs or derivatives could be fusion polypeptides, incorporating moieties which render purification easier, for example by effectively tagging the desired protein or polypeptide, it may be necessary to remove

the "tag" or it may be the case that the fusion polypeptide itself retains sufficient antigenicity to be useful.

It is well known that is possible to screen an antigenic polypeptide to identify epitopic regions, i.e. those regions which are responsible for the polypeptide's antigenicity or immunogenicity. Methods for carrying out such screening are well known in the art. Thus, the fragments of the present invention should include one or more such epitopic regions or be sufficiently similar to such regions to retain their antigenic/immunogenic properties.

Thus, for fragments according to the present invention the degree of identity is perhaps irrelevant, since they may be 100% identical to a particular part of a polypeptide, analog as described herein.

Thus, what is important for analogs, derivatives and fragments is that they possess at least a degree of the antigenicity/immunogenicity of the protein or polypeptide from which they are derived.

Also included are polypeptides which have fused thereto other compounds which alter the polypeptides biological or pharmacological properties i.e. polyethylene glycol (PEG) to increase half-life; leader or secretory amino acid sequences for ease of purification; prepro- and pro- sequences; and (poly)saccharides.

Furthermore, in those situations where amino acid regions are found to be polymorphic, it may be desirable to vary one or more particular amino acids to more effectively mimic the different epitopes of the different streptococcus strains.

Moreover, the polypeptides of the present invention can be modified by terminal -NH_2 acylation (eg. by acetylation, or thioglycolic acid amidation, terminal carboxy amidation, e.g. with ammonia or methylamine) to provide stability, increased hydrophobicity for linking or binding to a support or other molecule.

Also contemplated are hetero and homo polypeptide multimers of the polypeptide fragments and analogues. These polymeric forms include, for example, one or more polypeptides that have been cross-linked with cross-linkers such as avidin/biotin, gluteraldehyde or dimethylsuperimidate. Such polymeric forms also include polypeptides containing two or more tandem or inverted contiguous sequences, produced from multicistronic mRNAs generated by recombinant DNA technology. In a further embodiment, the present invention also relates to chimeric polypeptides which comprise one or more polypeptides or fragments or analogs thereof as defined in the figures of the present application.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides having a sequence chosen from SEQ ID NO: 2, or fragments or analogs thereof; provided that the polypeptides are linked as to formed a chimeric polypeptide.

In a further embodiment, the present invention also relates to chimeric polypeptides comprising two or more polypeptides having a sequence chosen from SEQ ID NO: 2 provided that the polypeptides are linked as to formed a chimeric polypeptide.

Preferably, a fragment, analog or derivative of a polypeptide of the invention will comprise at least one antigenic region i.e. at least one epitope.

In order to achieve the formation of antigenic polymers (i.e. synthetic multimers), polypeptides may be utilized having bishaloacetyl groups, nitroarylhalides, or the like, where the reagents being specific for thio groups. Therefore, the link between two mercapto groups of the different polypeptides may be a single bond or may be composed of a linking group of at least two, typically at least four, and not more than 16, but usually not more than about 14 carbon atoms.

In a particular embodiment, polypeptide fragments and analogs of the invention do not contain a methionine (Met) starting

residue. Preferably, polypeptides will not incorporate a leader or secretory sequence (signal sequence). The signal portion of a polypeptide of the invention may be determined according to established molecular biological techniques. In general, the polypeptide of interest may be isolated from a streptococcal culture and subsequently sequenced to determine the initial residue of the mature protein and therefore the sequence of the mature polypeptide.

It is understood that polypeptides can be produced and/or used without their start codon (methionine or valine) and/or without their leader peptide to favor production and purification of recombinant polypeptides. It is known that cloning genes without sequences encoding leader peptides will restrict the polypeptides to the cytoplasm of E. coli and will facilitate their recovery (Glick, B.R. and Pasternak, J.J. (1998) Manipulation of gene expression in prokaryotes. In "Molecular biotechnology: Principles and applications of recombinant DNA", 2nd edition, ASM Press, Washington DC, p.109-143).

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polypeptide of the invention, together with a carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iii) a vaccine comprising a polypeptide of the invention and a carrier, diluent or adjuvant; (iv) a method for inducing an immune response against Streptococcus, in a host, by administering to the host, an immunogenically effective amount of a polypeptide of the invention to elicit an immune response, e.g., a protective immune response to Streptococcus; and particularly, (v) a method for preventing and/or treating a Streptococcus infection, by administering a prophylactic or therapeutic amount of a polypeptide of the invention to a host in need.

According to another aspect of the invention, there are also provided (i) a composition of matter containing a polynucleotide of the invention, together with a carrier, diluent or adjuvant; (ii) a pharmaceutical composition comprising a polynucleotide of the invention and a carrier, diluent or adjuvant; (iii) a method

for inducing an immune response against Streptococcus, in a host, by administering to the host, an immunogenically effective amount of a polynucleotide of the invention to elicit an immune response, e.g., a protective immune response to Streptococcus; and particularly, (iv) a method for preventing and/or treating a Streptococcus infection, by administering a prophylactic or therapeutic amount of a polynucleotide of the invention to a host in need.

Before immunization, the polypeptides of the invention can also be coupled or conjugated to carrier proteins such as tetanus toxin, diphtheria toxin, hepatitis B virus surface antigen, poliomyelitis virus VP1 antigen or any other viral or bacterial toxin or antigen or any suitable proteins to stimulate the development of a stronger immune response. This coupling or conjugation can be done chemically or genetically. A more detailed description of peptide-carrier conjugation is available in Van Regenmortel, M.H.V., Briand J.P., Muller S., Plaué S., «Synthetic Polypeptides as antigens» in Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 19 (ed.) Burdou, R.H. & Van Knippenberg P.H. (1988), Elsevier New York.

According to another aspect, there are provided pharmaceutical compositions comprising one or more Streptococcal polypeptides of the invention in a mixture with a pharmaceutically acceptable adjuvant. Suitable adjuvants include (1) oil-in-water emulsion formulations such as MF59™, SAF™, Ribi™ ; (2) Freund's complete or incomplete adjuvant; (3) salts i.e. $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)_2$, $Al(OH)_3$, $AlPO_4$, silica, kaolin; (4) saponin derivatives such as Stimulon™ or particles generated therefrom such as ISCOMs (immunostimulating complexes); (5) cytokines such as interleukins, interferons, macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF) ; (6) other substances such as carbon polynucleotides i.e. poly IC and poly AU, detoxified cholera toxin (CTB)and E.coli heat labile toxin for induction of mucosal immunity. A more detailed description of adjuvant is available in a review by M.Z.I Khan et al. in Pharmaceutical Research, vol. 11, No. 1 (1994) pp2-11, and also in another review by Gupta et al., in Vaccine, Vol. 13, No. 14, pp1263-1276 (1995) and in WO 99/24578, which are herein

incorporated by reference. Preferred adjuvants include QuilA™, QS21™, Alhydrogel™ and Adjuphos™.

Pharmaceutical compositions of the invention may be administered parenterally by injection, rapid infusion, nasopharyngeal absorption, dermoabsorption, or buccal or oral.

Pharmaceutical compositions of the invention are used for the treatment or prophylaxis of streptococcal infection and/or diseases and symptoms mediated by streptococcal infection as described in P.R. Murray (Ed, in chief),E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken. Manual of Clinical Microbiology, ASM Press, Washington, D.C. sixth edition, 1995, 1482p which are herein incorporated by reference. In one embodiment, pharmaceutical compositions of the present invention are used for the prophylaxis or treatment of pharyngitis, erysipelas and impetigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis and also toxic shock. In one embodiment, pharmaceutical compositions of the invention are used for the prophylaxis or treatment of Streptococcus infection and/or diseases and symptoms mediated by Streptococcus infection, in particular group A Streptococcus (Streptococcus pyogenes), group B Streptococcus (GBS or S.agalactiae), S.pneumoniae, S.dysgalactiae, S.uberis, S.nocardia as well as Staphylococcus aureus. In a further embodiment, the Streptococcus infection is S. pyogenes.

In a further embodiment, the invention provides a method for prophylaxis or treatment of Streptococcus infection in a host susceptible to Streptococcus infection comprising administering to said host a therapeutic or prophylactic amount of a composition of the invention.

As used in the present application, the term "host" includes mammals. In a further embodiment, the mammal is human.

In a particular embodiment, pharmaceutical compositions are administered to those hosts at risk of streptococcus infection such as infants, elderly and immunocompromised hosts.

Pharmaceutical compositions are preferably in unit dosage form of about 0.001 to 100 µg/kg (antigen/body weight) and more preferably 0.01 to 10 µg/kg and most preferably 0.1 to 1 µg/kg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

Pharmaceutical compositions are preferably in unit dosage form of about 0.1 µg to 10 mg and more preferably 1µg to 1 mg and most preferably 10 to 100 µg 1 to 3 times with an interval of about 1 to 6 week intervals between immunizations.

According to another aspect, there are provided polynucleotides encoding polypeptides characterized by the amino acid sequence comprising SEQ ID NO: 2 or fragments or analogs thereof.

In one embodiment, polynucleotides are those illustrated in SEQ ID No: 1 which may include the open reading frames (ORF), encoding the polypeptides of the invention.

It will be appreciated that the polynucleotide sequences illustrated in the figures may be altered with degenerate codons yet still encode the polypeptides of the invention. Accordingly the present invention further provides polynucleotides which hybridize to the polynucleotide sequences herein above described (or the complement sequences thereof) having 50% identity between sequences. In one embodiment, at least 70% identity between sequences. In one embodiment, at least 75% identity between sequences. In one embodiment, at least 80% identity between sequences. In one embodiment, at least 85% identity between sequences. In one embodiment, at least 90% identity between sequences. In a further embodiment, polynucleotides are hybridizable under stringent conditions i.e. having at least 95% identity. In a further embodiment, more than 97% identity.

Suitable stringent conditions for hybridation can be readily determined by one of skilled in the art (see for example Sambrook et al., (1989) Molecular cloning : A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y.; Current Protocols in Molecular

Biology, (1999) Edited by Ausubel F.M. et al., John Wiley & Sons, Inc., N.Y.).

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises SEQ ID NO: 2, or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises SEQ ID NO: 2.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising SEQ ID NO: 2, or fragments or analogs thereof.

In a further embodiment, the present invention provides polynucleotides that hybridize under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising SEQ ID NO: 2.

In a further embodiment, polynucleotides are those encoding polypeptides of the invention illustrated in SEQ ID NO: 2 or fragments or analogs thereof.

In a further embodiment, polynucleotides are those illustrated in SEQ ID NO: 1 encoding polypeptides of the invention or fragments or analogs thereof.

In a further embodiment, polynucleotides are those encoding polypeptides of the invention illustrated in SEQ ID NO: 2.

In a further embodiment, polynucleotides are those illustrated in SEQ ID NO: 1 encoding polypeptides of the invention.

As will be readily appreciated by one skilled in the art, polynucleotides include both DNA and RNA.

The present invention also includes polynucleotides complementary to the polynucleotides described in the present application.

In a further aspect, polynucleotides encoding polypeptides of the invention, or fragments, analogs or derivatives thereof, may be used in a DNA immunization method. That is, they can be incorporated into a vector which is replicable and expressible upon injection thereby producing the antigenic polypeptide in vivo. For example polynucleotides may be incorporated into a plasmid vector under the control of the CMV promoter which is functional in eukaryotic cells. Preferably the vector is injected intramuscularly.

According to another aspect, there is provided a process for producing polypeptides of the invention by recombinant techniques by expressing a polynucleotide encoding said polypeptide in a host cell and recovering the expressed polypeptide product. Alternatively, the polypeptides can be produced according to established synthetic chemical techniques i.e. solution phase or solid phase synthesis of oligopeptides which are ligated to produce the full polypeptide (block ligation).

General methods for obtention and evaluation of polynucleotides and polypeptides are described in the following references: Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York; PCR Cloning Protocols, from Molecular Cloning to Genetic Engineering, Edited by White B.A., Humana Press, Totowa, New Jersey, 1997, 490 pages; Protein Purification, Principles and Practices, Scopes R.K., Springer-Verlag, New York, 3rd Edition, 1993, 380 pages; Current Protocols in Immunology, Edited by Coligan J.E. et al., John Wiley & Sons Inc., New York which are herein incorporated by reference.

For recombinant production, host cells are transfected with vectors which encode the polypeptide, and then cultured in a nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes. Suitable vectors are those that are viable and replicable in the chosen host and include chromosomal, non-chromosomal and synthetic DNA sequences e.g. bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA. The polypeptide sequence may be incorporated in the vector at the appropriate site using restriction enzymes such that it is operably linked to an expression control region comprising a promoter, ribosome binding site (consensus region or Shine-Dalgarno sequence), and optionally an operator (control element). One can select individual components of the expression control region that are appropriate for a given host and vector according to established molecular biology principles (Sambrook et al, Molecular Cloning: A Laboratory Manual, 2nd ed, Cold Spring Harbor, N.Y., 1989; Current Protocols in Molecular Biology, Edited by Ausubel F.M. et al., John Wiley and Sons, Inc. New York incorporated herein by reference). Suitable promoters include but are not limited to LTR or SV40 promoter, E.coli lac, tac or trp promoters and the phage lambda P_L promoter. Vectors will preferably incorporate an origin of replication as well as selection markers i.e. ampicilin resistance gene. Suitable bacterial vectors include pET, pQE70, pQE60, pQE-9, pD10 phagescript, psiX174, pbluescript SK, pbsks,

pNH8A, pNH16a, pNH18A, pNH46A, ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 and eukaryotic vectors pBlueBacIII, pWLNEO, pSV2CAT, pOG44, pXT1, pSG, pSVK3, pBPV, pMSG and pSVL. Host cells may be bacterial i.e. E.coli, Bacillus subtilis, Streptomyces; fungal i.e. Aspergillus niger, Aspergillus nidulins; yeast i.e. Saccharomyces or eukaryotic i.e. CHO, COS.

Upon expression of the polypeptide in culture, cells are typically harvested by centrifugation then disrupted by physical or chemical means (if the expressed polypeptide is not secreted into the media) and the resulting crude extract retained to isolate the polypeptide of interest. Purification of the polypeptide from culture media or lysate may be achieved by established techniques depending on the properties of the polypeptide i.e. using ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, hydroxylapatite chromatography and lectin chromatography. Final purification may be achieved using HPLC.

The polypeptides may be expressed with or without a leader or secretion sequence. In the former case the leader may be removed using post-translational processing (see US 4,431,739; US 4,425,437; and US 4,338,397 incorporated herein by reference) or be chemically removed subsequent to purifying the expressed polypeptide.

According to a further aspect, the streptococcal polypeptides of the invention may be used in a diagnostic test for Streptococcus infection, in particular S. pyogenes infection. Several diagnostic methods are possible, for example detecting Streptococcus organism in a biological sample, the following procedure may be followed:

a) obtaining a biological sample from a host;

b) incubating an antibody or fragment thereof reactive with a Streptococcus polypeptide of the invention with the biological sample to form a mixture; and

c) detecting specifically bound antibody or bound fragment in the mixture which indicates the presence of Streptococcus.

Alternatively, a method for the detection of antibody specific to a Streptococcus antigen in a biological sample containing or suspected of containing said antibody may be performed as follows:

a) obtaining a biological sample from a host;
b) incubating one or more Streptococcus polypeptides of the invention or fragments thereof with the biological sample to form a mixture; and
c) detecting specifically bound antigen or bound fragment in the mixture which indicates the presence of antibody specific to Streptococcus.

One of skill in the art will recognize that this diagnostic test may take several forms, including an immunological test such as an enzyme-linked immunosorbent assay (ELISA), a radioimmunoassay or a latex agglutination assay, essentially to determine whether antibodies specific for the protein are present in an organism.

The DNA sequences encoding polypeptides of the invention may also be used to design DNA probes for use in detecting the presence of Streptococcus in a biological sample suspected of containing such bacteria. The detection method of this invention comprises:

a) obtaining the biological sample from a host;
b) incubating one or more DNA probes having a DNA sequence encoding a polypeptide of the invention or fragments thereof with the biological sample to form a mixture; and
c) detecting specifically bound DNA probe in the mixture which indicates the presence of Streptococcus bacteria.

The DNA probes of this invention may also be used for detecting circulating Streptococcus i.e. S. pyogenes nucleic acids in a sample, for example using a polymerase chain reaction, as a method of diagnosing Streptococcus infections. The probe may be synthesized using conventional techniques and may be immobilized

on a solid phase, or may be labelled with a detectable label. A preferred DNA probe for this application is an oligomer having a sequence complementary to at least about 6 contiguous nucleotides of the S. pyogenes polypeptides of the invention.

Another diagnostic method for the detection of Streptococcus in a host comprises:

a) labelling an antibody reactive with a polypeptide of the invention or fragment thereof with a detectable label;
b) administering the labelled antibody or labelled fragment to the host; and
c) detecting specifically bound labelled antibody or labelled fragment in the host which indicates the presence of Streptococcus.

According to one aspect, the present invention provides the use of an antibody for treatment and/or prophylaxis of streptococcal infections.

A further aspect of the invention is the use of the Streptococcus polypeptides of the invention as immunogens for the production of specific antibodies for the diagnosis and in particular the treatment of streptococcus infection. Suitable antibodies may be determined using appropriate screening methods, for example by measuring the ability of a particular antibody to passively protect against streptococcus infection in a test model. One example of an animal model is the mouse model described in the examples herein. The antibody may be a whole antibody or an antigen-binding fragment thereof and may belong to any immunoglobulin class. The antibody or fragment may be of animal origin, specifically of mammalian origin and more specifically of murine, rat or human origin. It may be a natural antibody or a fragment thereof, or if desired, a recombinant antibody or antibody fragment. The term recombinant antibody or antibody fragment means antibody or antibody fragment which was produced using molecular biology techniques. The antibody or antibody fragments may be polyclonal, or preferably monoclonal. It may be specific for a number of

epitopes associated with the S. pyogenes polypeptides but is preferably specific for one.

A further aspect of the invention is the use of the antibodies directed to the polypeptides of the invention for passive immunization. One could use the antibodies described in the present application.

A further aspect of the invention is a method for immunization, whereby an antibody raised by a polypeptide of the invention is administered to a host in an amount sufficient to provide a passive immunization.

In a further embodiment, the invention provides the use of a pharmaceutical composition in the manufacture of a medicament for the prophylactic or therapeutic treatment of streptococcal infection.

In a further embodiment, the invention provides a kit comprising a polypeptide of the invention for detection or diagnosis of streptococcal infection.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLE 1

This example illustrates the cloning and molecular characteristics of BVH-P7 gene and corresponding polypeptide.

The coding region of S. pyogenes BVH-P7 (SEQ ID NO: 1) gene was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, CA) from genomic DNA of serotype M1 S.

pyogenes strain ATCC700294 using the following oligonucleotide primers that contained base extensions for the addition of restriction sites *Nde*I (CATATG) and *Not*I (GCGGCCGC): DMAR293 (SEQ ID NO: 9) and DMAR294 (SEQ ID NO: 10), which are presented in Table 1. PCR products were purified from agarose gel using a QIAquick gel extraction kit from QIAgen following the manufacturer's instructions (Chatsworth, CA), and digested with *Nde*I and *Not*I (Amersham Pharmacia Biotech Inc, Baie D'Urfé, Canada). The pET-21b(+) vector (Novagen, Madison, WI) was digested with *Nde*I and *Not*I and purified from agarose gel using a QIAquick gel extraction kit from QIAgen (Chatsworth, CA). The *Nde*I-*Not*I PCR products were ligated to the *Nde*I-*Not*I pET-21b(+) expression vector. The ligated products were transformed in E. coli strain DH5•[Φ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *hsd*R17(r_K-m_K+) *deo*R *thi*-1 *sup*E44 λ⁻ *gyr*A96 *rel*A1] (Gibco BRL, Gaithersburg, MD) according to the method of Simanis (Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). Recombinant pET-21b(+) plasmid (rpET21b(+)) containing BVH-P7 gene was purified using a QIAgen plasmid kit (Chatsworth, CA) and DNA insert was sequenced (Taq Dye Deoxy Terminator Cycle Sequencing kit, ABI, Foster City, CA).

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Table 1. Oligonucleotide primers used for PCR amplifications of S. pyogenes BVH-P7 gene

Genes	Primers I.D. (SEQ ID NO)	Restriction site	Vector	Sequence
BVH-P7	DMAR293 (3) (SEQ ID NO: 9)	*NdeI*	pET21b	5'-GTAGTCACCCACCATATGGAAGTTTTTAG-3'
BVH-P7	DMAR294 (4) (SEQ ID NO: 10)	*NotI*	pET21b	5'-TTTTTTCTTTGCGGCCGCAGTTATTAGT-3'
BVH-P7	DMAR480a (5) (SEQ ID NO: 11)	*BamHI*	pCMV-GH	5'-GGGGATCCCACCCACAATCAGG-3'
BVH-P7	DMAR481a (6) (SEQ ID NO: 12)	*SalI*	pCMV-GH	5'-GGTTGTCGACAGTAAAGCAACGCTAGTG-3'

It was determined that the 3027-bp including a stop codon (TAA) open reading frame (ORF) of BVH-P7 encodes a 1008 amino-acid-residues polypeptide with a predicted pI of 6.18 and a predicted molecular mass of 111,494.44 Da. Analysis of the predicted amino acid residues sequence (SEQ ID NO: 2) using the PSORTII software (Real World Computing Partnership (http://psort.nibb.ac.jp)) suggested the existence of a 21 amino acid residues signal peptide (MKKHLKTVALTLTTVSVVTHN), which ends with a cleavage site

situated between an asparagine and a glutamine residues. Analysis of the amino-acid-residues sequence revealed the presence of a cell wall anchoring motif (LPXTGX) located between residues 974 and 981.

To confirm the presence by PCR amplification of BVH-P7 (SEQ ID NO: 1) gene, the following 4 serologically distinct S. pyogenes strains were used: the serotype M1 S. pyogenes strain ATCC700294 and the serotype M3 S. pyogenes strain ATCC12384 were obtained from the American Type Culture Collection (Rockville, MD); the serotype M6 S. pyogenes SPY67 clinical isolate was provided by the Centre de recherche en infectiologie du Centre hospitalier de l'université Laval, Sainte-Foy; and S. pyogenes strain B514 which was initially isolated from a mouse was provided by Susan Hollingshead, from University of Alabama, Birmingham. The E. coli strain XL1-Blue MRF' was used in these experiments as negative control. Chromosomal DNA was isolated from each S. pyogenes strain as previously described (Jayarao BM et al. 1991. J. Clin. Microbiol. 29:2774-2778). BVH-P7 (SEQ ID NO: 1) gene was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, CA) from the genomic DNA purified from the 4 S. pyogenes strains, and the control E. coli strain using the oligonucleotide primers DMAR293 (SEQ ID NO: 9) and DMAR294 (SEQ ID NO: 10) (Table 1). PCR was performed with 30 cycles of 45 sec at 95°C, 45 sec at 50°C and 2 min at 72°C and a final elongation period of 7 min at 72°C. The PCR products were size fractionated in 1% agarose gels and were visualized by ethidium bromide staining. The results of these PCR amplifications are presented in Table 2. The analysis of the amplification products revealed that BVH-P7 (SEQ ID NO: 1) gene was present in the genome of all of the 4 S. pyogenes strains tested. No such product was detected

when the control E. coli DNA was submitted to identical PCR amplifications with these oligonucleotide primers.

Table 2. Identification of S. pyogenes BVH-P7 gene by PCR amplification in the genome of four serologically distinct S. pyogenes strains

Strain Identification	Identification of BVH-P7 gene
ATCC700294 (M1)	+
ATCC12384 (M3)	+
SPY67 (M6)	+
B514*	+
E. coli XL1 Blue MRF'	-

* Mouse isolate

EXAMPLE 2

This example illustrates the cloning of S. pyogenes BVH-P7 gene in CMV plasmid pCMV-GH.

The DNA coding region of S. pyogenes protein was inserted in phase downstream of a human growth hormone (hGH) gene which was under the transcriptional control of the cytomegalovirus (CMV) promotor in the plasmid vector pCMV-GH (Tang et al., Nature, 1992, 356:152). The CMV promotor is a non functional plasmid in E. coli cells but active upon administration of the plasmid in eukaryotic cells. The vector also incorporated the ampicillin resistance gene.

The coding regions of BVH-P7 (SEQ ID NO: 1) gene without its leader peptide region was amplified by PCR (Robocycler Gradient 96 Temperature cycler, Stratagene, LaJolla, CA) from genomic DNA of serotype M1 S. pyogenes strain ATCC700294 using oligonucleotide primers DMAR480a (SEQ ID NO: 11) and DMAR481a (SEQ ID NO: 12) that contained base extensions for the addition of restriction sites *Bam*HI (GGATCC) and *Sal*I (GTCGAC) which are described in Table 1. The PCR products were purified from agarose gel using a QIAquick gel extraction kit from QIAgen (Chatsworth, CA), digested with restriction enzymes (Amersham Pharmacia Biotech Inc, Baie d'Urfé, Canada). The pCMV-GH vector (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas) was digested with *Bam*HI and *Sal*I and purified from agarose gel using the QIAquick gel extraction kit from QIAgen (Chatsworth, CA). The *Bam*HI-*Sal*I DNA fragment was ligated to the *Bam*HI-*Sal*I-pCMV-GH vector to create the hGH-BVH-P7 fusion protein under the control of the CMV promoter. The ligated product was transformed into E. coli strain DH5• [ϕ80d*lac*ZΔM15 Δ(*lac*ZYA-*arg*F)U169 *end*A1 *rec*A1 *hsd*R17(r_K-m_K+) *deo*R *thi*-1 *sup*E44 λ⁻ *gyr*A96 *rel*A1] (Gibco BRL, Gaithersburg, MD) according to the method of Simanis (Hanahan, D. DNA Cloning, 1985, D.M. Glover (ed), pp. 109-135). The recombinant pCMV plasmid was purified using a QIAgen plasmid kit (Chatsworth, CA) and the nucleotide sequence of the DNA insert was verified by DNA sequencing.

EXAMPLE 3

This example illustrates the use of DNA to elicit an immune response to S. pyogenes BVH-P7 protein antigen.

Groups of 8 female BALB/c mice (Charles River, St-Constant, Québec, Canada) were immunized by intramuscular injection of 100 µl three times at two- or three-week intervals with 50 µg of

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recombinant pCMV-GH encoding BVH-P7 (SEQ ID NO: 1) gene in presence of 50 µg of granulocyte-macrophage colony-stimulating factor (GM-CSF)-expressing plasmid pCMV-GH-GM-CSF (Laboratory of Dr. Stephen A. Johnston, Department of Biochemistry, The University of Texas, Dallas, Texas). As control, groups of mice were injected with 50 µg of pCMV-GH in presence of 50 µg of pCMV-GH-GM-CSF. Blood samples were collected from the orbital sinus prior to each immunization and seven days following the third injection and serum antibody responses were determined by ELISA using the BVH-P7 His-tagged labeled S. pyogenes recombinant protein as coating antigen. The production and purification of this BVH-P7 His-tagged labeled S. pyogenes recombinant protein is presented in Example 4.

EXAMPLE 4

This example illustrates the production and purification of S. pyogenes BVH-P7 recombinant protein.

The recombinant pET-21b(+)plasmid with BVH-P7 (SEQ ID NO: 1) gene was used to transform by electroporation (Gene Pulser II apparatus, BIO-RAD Labs, Mississauga, Canada) E. coli strain Tuner (DE3) (F^-ompT $hsdS_B$ ($r^-_Bm^-_B$) gal dcm lacYI (DE3)) (Novagen, Madison, WI). In this strain of E. coli, the T7 promotor controlling expression of the recombinant protein is specifically recognized by the T7 RNA polymerase (present on the λDE3 prophage) whose gene is under the control of the lac promotor which is inducible by isopropyl-ß-d-thio-galactopyranoside (IPTG). The transformants Tuner (DE3)/rpET21 (+) were grown at 37°C with agitation at 250 rpm in LB broth (peptone 10g/L, yeast extract 5g/L, NaCl 10g/L) containing 100 µg of carbenicillin (Sigma-Aldrich Canada Ltd., Oakville, Canada) per ml until the A_{600} reached a value of 0.6. In order to induce the production of BVH-P7 His-tagged S. pyogenes recombinant protein, the cells were incubated for 3 additional hours in the presence of IPTG at a final concentration of 0.1 mM. Induced cells from a 500 ml culture were pelleted by centrifugation and frozen at -70°C.

The purification of the BVH-P7 His-tagged recombinant protein

from the non-soluble fraction of IPTG-induced Tuner (DE3)/rpET21b(+) was done by affinity chromatography based on the properties of the His•Tag sequence (6 consecutive histidine residues) to bind to divalent cations (Ni^{2+}) immobilized on the His•Bind metal chelation resin. Briefly, the pelleted cells obtained from a 500 mL culture induced with IPTG was resuspended in lysis buffer (20 mM Tris, 500 mM NaCl, 10 mM imidazole, pH 7.9) containing 6M Guanidine-HCl, sonicated and centrifuged at 12,000 X g for 20 min to remove debris. The supernatant was incubated with Ni-NTA agarose resin (Qiagen, Mississauga, Ontario, Canada) for 45 min at 4°C. The BVH-P7 His-tagged S. pyogenes recombinant protein was eluted from the resin with a solution containing 6M Guanidine-HCl and 250 mM imidazole-500mM NaCl-20 mM Tris, pH 7.9. The removal of the salt and imidazole from the samples was done by dialysis against 10mM Tris and 0.9% NaCl, pH 7.9 overnight at 4°C. The amount of recombinant protein was estimated by MicroBCA (Pierce, Rockford, Illinois).

EXAMPLE 5

This example illustrates the reactivity of the BVH-P7 His-tagged S. pyogenes recombinant protein with human sera and sera collected from mice after immunization with S. pyogenes antigenic preparations.

As shown in Table 3, purified His-tagged BVH-P7 recombinant protein was recognized in immunoblots by the antibodies present in the pool of normal sera. This is an important result since it clearly indicates that human which are normally in contact with S. pyogenes do develop antibodies that are specific to that protein. These particular human antibodies might be implicated in the protection against S. pyogenes infection. In addition, immunoblots also revealed that sera collected from mice immunized with S. pyogenes antigenic preparations enriched membrane proteins which protected mice against lethal challenge also developed antibodies that recognized BVH-P7 His-tagged recombinant protein. This result indicates that this protein was present in S. pyogenes antigenic preparation that protected mice against infection and that this streptococcal protein induced

antibodies that reacted with the corresponding His-tagged recombinant protein.

Table 3. Reactivity in immunoblots of human sera and sera collected from mice after immunization with S. pyogenes antigenic preparations with BVH-P7 His-tagged recombinant protein.

Purified recombinant protein I.D.[1]	Apparent molecular weight (kDa)[2]	Reactivity in immunoblots with	
		Human sera[3]	Mouse sera[4]
BVH-P7	110	+	+

[1]BVH-P7 His-tagged recombinant protein produced and purified as described in Example 7 was used to perform the immunoblots.

[2]Molecular weight of the BVH-P7 His-tagged recombinant protein was estimated after SDS-PAGE.

[3]Two sera collected from healthy human volunteers were pooled together and diluted 1/500 to perform the immunoblots.

[4]Mouse sera collected after immunization with S. pyogenes antigenic preparations enriched membrane proteins were pooled and diluted 1/500 to perform the immunoblots. These mice were protected against a lethal S. pyogenes challenge.

EXAMPLE 6

This example illustrates the accessibility to antibodies of the S. pyogenes BVH-P7 protein at the surface of intact streptococcal cells.

Bacteria were grown in Tood Hewitt (TH) broth (Difco Laboratories, Detroit, MI) with 0.5% Yeast extract (Difco Laboratories) and 0.5% peptone extract (Merck, Darmstadt, Germany) at 37°C in a 8% CO_2 atmosphere to give an OD_{490nm} of 0.600 (~10^8 CFU/ml). Dilutions of anti-BVH-P7 or control sera were then added and allowed to bind to the cells, which were incubated for 2 h at 4°C. Samples were washed 4 times in blocking buffer [phosphate-buffered saline (PBS) containing 2% bovine serum albumin (BSA)], and then 1 ml of goat fluorescein (FITC)-

conjugated anti-mouse IgG + IgM diluted in blocking buffer was added. After an additional incubation of 60 min at room temperature, samples were washed 4 times in blocking buffer and fixed with 0.25 % formaldehyde in PBS buffer for 18-24 h at 4°C. Cells were washed 2 times in PBS buffer and resuspended in 500 µl of PBS buffer. Cells were kept in the dark at 4°C until analyzed by flow cytometry (Epics® XL; Beckman Coulter, Inc.). Ten thousands intact S. pyogenes cells were analyzed per sample and the results were expressed as percentage of labeled cells and fluorescence index. The fluorescence index was calculated as the median fluorescence value obtained after labeling the streptococcal cells with an immune serum divided by the fluorescence value obtained for a control mouse serum. A fluorescence value of 1 indicated that there was no binding of antibodies at the surface of intact streptococcal cells.

Sera collected from eight mice immunized with BVH-P7 His-tagged recombinant protein were analyzed by cytofluorometry and the results are presented in Table 4. All of the sera collected from mice immunized with purified BVH-P7 His-tagged protein contained BVH-P7-specific antibodies that efficiently recognized their corresponding surface exposed epitopes on the heterologous (ATCC12384; serotype M3) S. pyogenes strain tested. The fluorescence index varied from 10 to 18. It was determined that more than 97 % of the 10,000 S. pyogenes cells analyzed were labeled with the antibodies present in the BVH-P7 specific anti-sera. These sera were also pooled and reacted with the following S. pyogenes strains: serotype M1 S. pyogenes strain ATCC 700294, serotype M3 and serotype M18 S. pyogenes strain ATCC12357 were obtained from the American Type Culture Collection (Rockville, MD, USA); the serotype M6 S. pyogenes SPY69 and M2 S. pyogenes SPY68 clinical isolates were provided by the Centre de recherche en infectiologie du Centre hospitalier de l'université Laval, Sainte-Foy. The BVH-P7-specific antibodies present in the pool of sera collected after immunization with the purified His-tagged recombinant BVH-P7 protein attached at the bacterial surface of each of these streptococcal strains with fluorescence index between 4 up to 9. On the contrary, no labeling of the streptococcal cells were

noted when pools of sera collected from unimmunized or sham-immunized mice were used. These observations clearly demonstrate that the BVH-P7 protein is accessible at the surface where it can be easily recognized by antibodies. Anti-S. pyogenes antibodies were shown to play an important role in the protection against S. pyogenes infection.

Table 4. Evaluation of the attachment of BVH-P7-specific antibodies at the surface of intact cells of S. pyogenes ATCC12384 strain (serotype M3).

Serum Identification	Fluorescence Index[2]	% of labeled cells[3]
S1[1]	11	97
S2	11	97
S3	13	98
S4	16	99
S5	10	97
S6	12	97
S7	13	98
S8	18	99
Pool of negative control sera[4]	1	9
Positive control serum[5]	12	98

[1] The mice S1 to S8 were injected subcutaneously three times at three-week intervals with 20 µg of purified BVH-P7 recombinant protein mixed with 10 µg of QuilA adjuvant (Cedarlane Laboratories, Hornby, Canada). The sera were diluted 1/50.

[2] The fluorescence index was calculated as the median fluorescence value obtained after labeling the streptococcal cells with an immune serum divided by the fluorescence value obtained for a control mouse serum. A fluorescence value of 1 indicated that there was no binding of antibodies at the surface of intact streptococcal cells.

[3] % of streptococcal labeled cells out of the 10,000 cells analyzed.

[4] Sera collected from unimmunized or sham-immunized mice were pooled diluted 1/50 and used as negative controls for this assay.

serum obtained from a mouse immunized with 20 μg of purified streptococcal recombinant M protein, a well known surface protein, was diluted 1/200 and was used as a positive control for the assay.

EXAMPLE 7

This example illustrates the protection against fatal S. pyogenes infection induced by passive immunization of mice with rabbit hyper-immune sera.

New Zealand rabbits (Charles River laboratories, St-Constant, Canada) were injected subcutaneously at multiple sites with 50 μg and 100 μg of the BVH-P7 His-tagged recombinant protein that was produced and purified as described in Example 4 and adsorbed to Alhydrogel adjuvant (Superfos Biosector a/s). Rabbits were immunized three times at three-week intervals with the BVH-P7 His-tagged recombinant protein. Blood samples were collected three weeks after the third injection. The antibodies present in the serum were purified by precipitation using 40% saturated ammonium sulfate. Groups of 10 female CD-1 mice (Charles River) were injected intravenously with 500 μl of purified serum collected from rabbits immunized with the BVH-P7 His-tagged recombinant protein, or rabbits immunized with an unrelated control recombinant protein. Eighteen hours later the mice were challenged with approximately $2x10^7$ CFU of the type 3 S. pyogenes strain ATCC12384. Samples of the S. pyogenes challenge inoculum were plated on blood agar plates to determine the CFU and to verify the challenge dose. Deaths were recorded for a period of 5 days.

EXAMPLE 8

This example illustrates the protection of mice against fatal S. pyogenes infection induced by immunization with purified recombinant BVH-P7 protein.

Groups of 8 female Balb/c mice (Charles River, St-Constant, Québec, Canada) were immunized subcutaneously three times at two-week intervals with 20 μg of affinity purified BVH-P7 His-

tagged recombinant protein in presence of 10 μg of QuilA adjuvant (Cedarlane Laboratories Ltd, Hornby, Canada) or, as control, with QuilA adjuvant alone in PBS. Blood samples were collected from the orbital sinus on day 1, 14 and 28 prior to each immunization and two weeks (day 42) following the third injection. One week later the mice were challenged with approximately $3x10^6$ CFU of the type 3 S. pyogenes strain ATCC 12384. Samples of the S. pyogenes challenge inoculum were plated on blood agar plates to determine the CFU and to verify the challenge dose. Deaths were recorded for a period of 7 days. Four of eight mice immunized with purified recombinant BVH-P7 protein were protected against the lethal challenge, compared to only 12 % (1/8) of mice which received the adjuvant alone (Table 1).

Table 5. Ability of recombinant BVH-P7 protein to elicit protection against GAS strain ATCC 12384 (Type 3).

Immunogen	No. mice surviving	% survival
20 μg BVH-P7 + 10% QuilA	4/8	50
QuilA adjuvant alone in PBS	1/8	12

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485 490 495

Leu Val Thr Ile Gly Asp Ala Ala Phe His Ile Asn His Ile Tyr Ala
500 505 510

Ile Val Leu Pro Glu Ser Val Gln Glu Ile Gly Arg Ser Ala Phe Arg
515 520 525

Gln Asn Gly Ala Asn Asn Leu Ile Phe Met Gly Ser Lys Val Lys Thr
530 535 540

Leu Gly Glu Met Ala Phe Leu Ser Asn Arg Leu Glu His Leu Asp Leu
545 550 555 560

Ser Glu Gln Lys Gln Leu Thr Glu Ile Pro Val Gln Ala Phe Ser Asp
565 570 575

Asn Ala Leu Lys Glu Val Leu Leu Pro Ala Ser Leu Lys Thr Ile Arg
580 585 590

Glu Glu Ala Phe Lys Lys Asn His Leu Lys Gln Leu Glu Val Ala Ser
595 600 605

Ala Leu Ser His Ile Ala Phe Asn Ala Leu Asp Asp Asn Asp Gly Asp
610 615 620

Glu Gln Phe Asp Asn Lys Val Val Val Lys Thr His His Asn Ser Tyr
625 630 635 640

Ala Leu Ala Asp Gly Glu His Phe Ile Val Asp Pro Asp Lys Leu Ser
645 650 655

Ser Thr Ile Val Asp Leu Glu Lys Ile Leu Lys Leu Ile Glu Gly Leu
660 665 670

Asp Tyr Ser Thr Leu Arg Gln Thr Thr Gln Thr Gln Phe Arg Asp Met
675 680 685

Thr Thr Ala Gly Lys Ala Leu Leu Ser Lys Ser Asn Leu Arg Gln Gly
690 695 700

Glu Lys Gln Lys Phe Leu Gln Glu Ala Gln Phe Phe Leu Gly Arg Val
705 710 715 720

Asp Leu Asp Lys Ala Ile Ala Lys Ala Glu Lys Ala Leu Val Thr Lys
725 730 735

Lys Ala Thr Lys Asn Gly Gln Leu Leu Glu Arg Ser Ile Asn Lys Ala
740 745 750

Val Leu Ala Tyr Asn Asn Ser Ala Ile Lys Lys Ala Asn Val Lys Arg
755 760 765

Leu Glu Lys Glu Leu Asp Leu Leu Thr Gly Leu Val Glu Gly Lys Gly
770 775 780

Pro Leu Ala Gln Ala Thr Met Val Gln Gly Val Tyr Leu Leu Lys Thr
785 790 795 800

Pro Leu Pro Leu Pro Glu Tyr Tyr Ile Gly Leu Asn Val Tyr Phe Asp
805 810 815

Lys Ser Gly Lys Leu Ile Tyr Ala Leu Asp Met Ser Asp Thr Ile Gly
820 825 830

Glu Gly Gln Lys Asp Ala Tyr Gly Asn Pro Ile Leu Asn Val Asp Glu
835 840 845

Asp Asn Glu Gly Tyr His Ala Leu Ala Val Ala Thr Leu Ala Asp Tyr
850 855 860

Glu Gly Leu Asp Ile Lys Thr Ile Leu Asn Ser Lys Leu Ser Gln Leu
865 870 875 880

Thr Ser Ile Arg Gln Val Pro Thr Ala Ala Tyr His Arg Ala Gly Ile
885 890 895

Phe Gln Ala Ile Gln Asn Ala Ala Ala Glu Ala Glu Gln Leu Leu Pro
900 905 910

Lys Ala Gly Thr His Ser Glu Lys Ser Ser Ser Ser Glu Ser Ala Asn
915 920 925

Ser Lys Asp Arg Gly Leu Gln Ser Asn Pro Lys Thr Asn Arg Gly Arg
930 935 940

His Ser Ala Ile Leu Pro Arg Thr Gly Ser Lys Gly Ser Phe Val Tyr
945 950 955 960

Gly Ile Leu Gly Tyr Thr Ser Val Ala Leu
965 970

<210> 5
<211> 963
<212> PRT
<213> Streptococcus pyogenes strain Spy69

<400> 5
Lys Gln Thr Gln Ala Ser Ser Ser Ile Ser Gly Ala Asp Tyr Ala Glu
1 5 10 15

Ser Ser Gly Lys Ser Lys Leu Lys Ile Asn Glu Thr Ser Gly Pro Val
20 25 30

Asp Asp Thr Val Thr Asp Leu Phe Ser Asp Lys Arg Thr Thr Pro Glu
35 40 45

Lys Ile Lys Asp Asn Leu Ala Lys Gly Pro Arg Glu Gln Glu Leu Lys
50 55 60

Ala Val Thr Glu Asn Thr Glu Ser Glu Lys Gln Ile Asn Ser Gly Ser
65 70 75 80

Gln Leu Glu Gln Ser Lys Glu Ser Leu Ser Leu Asn Lys Arg Val Pro
85 90 95

Ser Thr Ser Asn Trp Glu Ile Cys Asp Phe Ile Thr Lys Gly Asn Thr
100 105 110

Leu Val Gly Leu Ser Lys Ser Gly Val Glu Lys Leu Ser Gln Thr Asp
115 120 125

His Leu Val Leu Pro Ser Gln Ala Ala Asp Gly Thr Gln Leu Ile Gln
130 135 140

Val Ala Ser Phe Ala Phe Thr Pro Asp Lys Lys Thr Ala Ile Ala Glu
145 150 155 160

Tyr Thr Ser Arg Ala Gly Glu Asn Gly Glu Ile Ser Gln Leu Asp Val
165 170 175

Asp Gly Lys Glu Ile Ile Asn Glu Gly Glu Val Phe Asn Ser Tyr Leu
180 185 190

Leu Lys Lys Val Thr Ile Pro Thr Gly Tyr Lys His Ile Gly Gln Asp
195 200 205

Ala Phe Val Asp Asn Lys Asn Ile Ala Glu Val Asn Leu Pro Glu Ser
210 215 220

Leu Glu Thr Ile Ser Asp Tyr Ala Phe Ala His Leu Ala Leu Lys Gln
225 230 235 240

Ile Asp Leu Pro Asp Asn Leu Lys Ala Ile Gly Glu Leu Ala Phe Phe
245 250 255

Asp Asn Gln Ile Thr Gly Lys Leu Ser Leu Pro Arg Gln Leu Met Arg
260 265 270

Leu Ala Glu Arg Ala Phe Lys Ser Asn His Ile Lys Thr Ile Glu Phe
275 280 285

Arg Gly Asn Ser Leu Lys Val Ile Gly Glu Ala Ser Phe Gln Asp Asn
290 295 300

Asp Leu Ser Gln Leu Met Leu Pro Asp Gly Leu Glu Lys Ile Glu Ser
305 310 315 320

Glu Ala Phe Thr Gly Asn Pro Gly Asp Asp His Tyr Asn Asn Arg Val
325 330 335

Val Leu Trp Thr Lys Ser Gly Lys Asn Pro Tyr Gly Leu Ala Thr Glu
340 345 350

Asn Thr Tyr Val Asn Pro Asp Lys Ser Leu Trp Gln Glu Ser Pro Glu
355 360 365

Ile Asp Tyr Thr Lys Trp Leu Glu Glu Asp Phe Thr Tyr Gln Lys Asn
370 375 380

Ser Val Thr Gly Phe Ser Ser Lys Gly Leu Gln Lys Val Lys Arg Asn
385 390 395 400

Lys Asn Leu Glu Ile Pro Lys Gln His Asn Gly Val Thr Ile Thr Glu
405 410 415

Ile Gly Asp Asn Ala Phe Arg Asn Val Asn Phe Gln Asn Lys Thr Leu
420 425 430

Arg Lys Tyr Asp Leu Glu Glu Val Lys Leu Pro Ser Thr Ile Arg Lys
435 440 445

Ile Gly Ala Phe Ala Phe Gln Ser Asn Asn Leu Lys Ser Phe Glu Ala
450 455 460

Ser Asp Asp Leu Glu Glu Ile Lys Glu Gly Ala Phe Met Asn Asn Arg
465 470 475 480

Ile Glu Thr Leu Glu Leu Lys Asp Lys Leu Val Thr Ile Gly Asp Ala
485 490 495

Ala Phe His Ile Asn His Ile Tyr Ala Ile Val Leu Pro Glu Ser Val
500 505 510

Gln Glu Ile Gly Arg Ser Ala Phe Arg Gln Asn Gly Ala Asn Asn Leu
515 520 525

Ile Phe Met Gly Ser Lys Val Lys Thr Leu Gly Glu Met Ala Phe Leu
530 535 540

Ser Asn Arg Leu Glu His Leu Asp Leu Ser Glu Gln Lys Gln Leu Thr
545 550 555 560

Glu Ile Pro Val Gln Ala Phe Ser Asp Asn Ala Leu Lys Glu Val Leu
565 570 575

Leu Pro Ala Ser Leu Lys Thr Ile Arg Glu Glu Ala Phe Lys Lys Asn
580 585 590

His Leu Lys Gln Leu Glu Val Ala Ser Ala Leu Ser His Ile Ala Phe
595 600 605

Asn Ala Leu Asp Asp Asn Asp Gly Asp Glu Gln Phe Asp Asn Lys Val
610 615 620

Val Val Lys Thr His His Asn Ser Tyr Ala Leu Ala Asp Gly Glu His
625 630 635 640

Phe Ile Val Asp Pro Asp Lys Leu Ser Ser Thr Ile Val Asp Leu Glu
645 650 655

Lys Ile Leu Lys Leu Ile Glu Gly Leu Asp Tyr Ser Thr Leu Arg Gln
660 665 670

Thr Thr Gln Thr Gln Phe Arg Asp Met Thr Thr Ala Gly Lys Ala Leu
675 680 685

Leu Ser Lys Ser Asn Leu Arg Gln Gly Glu Lys Gln Lys Phe Leu Gln
690 695 700

Glu Ala Gln Phe Phe Leu Gly Arg Val Asp Leu Asp Lys Ala Ile Ala
705 710 715 720

Lys Ala Glu Lys Ala Leu Val Thr Lys Lys Ala Thr Lys Asn Gly Gln
725 730 735

Leu Leu Glu Arg Ser Ile Asn Lys Ala Val Ser Ala Tyr Asn Asn Ser
740 745 750

Ala Ile Lys Lys Ala Asn Val Lys Arg Leu Glu Lys Glu Leu Asp Leu
755 760 765

Leu Thr Gly Leu Val Glu Gly Lys Gly Pro Leu Ala Gln Ala Thr Met
770 775 780

Val Gln Gly Val Tyr Leu Leu Lys Thr Pro Leu Pro Leu Pro Glu Tyr
785 790 795 800

Tyr Ile Gly Leu Asn Val Tyr Phe Asp Lys Ser Gly Lys Leu Ile Tyr
805 810 815

Ala Leu Asp Met Ser Asp Thr Ile Gly Glu Gly Gln Lys Asp Ala Tyr
820 825 830

Gly Asn Pro Ile Leu Asn Val Asp Glu Asp Asn Glu Gly Tyr His Ala
835 840 845

Leu Ala Val Ala Thr Leu Ala Asp Tyr Glu Gly Leu Asp Ile Lys Thr
850 855 860

Ile Leu Asn Ser Lys Leu Ser Gln Leu Thr Ser Ile Arg Gln Val Pro
865 870 875 880

Thr Ala Ala Tyr His Arg Ala Gly Ile Phe Gln Ala Ile Gln Asn Ala
885 890 895

Ala Ala Glu Ala Glu Gln Leu Leu Pro Lys Pro Gly Thr His Ser Glu
900 905 910

Lys Ser Ser Ser Ser Glu Ser Ala Asn Ser Lys Asp Arg Gly Leu Gln
915 920 925

Ser Asn Pro Lys Thr Asn Arg Gly Arg His Ser Ala Ile Leu Pro Arg
930 935 940

Thr Gly Ser Lys Gly Ser Phe Val Tyr Gly Ile Leu Gly Tyr Thr Ser
945 950 955 960

Val Ala Leu

<210> 6
<211> 971
<212> PRT
<213> Streptococcus pyogenes strain Spy68

<400> 6
Leu Val Lys Glu Pro Ile Leu Lys Gln Thr Gln Ala Ser Ser Ser Ile
1 5 10 15

Ser Gly Ala Asp Tyr Ala Glu Ser Ser Gly Lys Ser Lys Leu Lys Ile
20 25 30

Asn Glu Thr Ser Gly Pro Val Asp Asp Thr Val Thr Asp Leu Phe Ser
35 40 45

Asp Lys Arg Thr Thr Pro Glu Lys Ile Lys Asp Asn Leu Ala Lys Gly
50 55 60

Pro Arg Glu Gln Glu Leu Lys Thr Val Thr Glu Asn Thr Glu Ser Glu
65 70 75 80

Lys Gln Ile Thr Ser Gly Ser Gln Leu Glu Gln Ser Lys Glu Ser Leu
85 90 95

Ser Leu Asn Lys Thr Val Pro Ser Thr Ser Asn Trp Glu Ile Cys Asp
100 105 110

Phe Ile Thr Lys Gly Asn Thr Leu Val Gly Leu Ser Lys Ser Gly Val
115 120 125

Glu Lys Leu Ser Gln Thr Asp His Leu Val Leu Pro Ser Gln Ala Ala
130 135 140

Asp Gly Thr Gln Leu Ile Gln Val Ala Ser Phe Ala Phe Thr Pro Asp
145 150 155 160

Lys Lys Thr Ala Ile Ala Glu Tyr Thr Ser Arg Ala Gly Glu Asn Gly
165 170 175

Glu Ile Ser Gln Leu Asp Val Asp Gly Lys Glu Ile Ile Asn Glu Gly
180 185 190

Glu Val Phe Asn Ser Tyr Leu Leu Lys Lys Val Thr Ile Pro Thr Gly
195 200 205

Tyr Lys His Ile Gly Gln Asp Ala Phe Val Asp Asn Lys Asn Ile Ala
210 215 220

Glu Val Asn Leu Pro Glu Ser Leu Glu Thr Ile Ser Asp Tyr Ala Phe
225 230 235 240

Ala His Leu Ala Leu Lys Gln Ile Asp Leu Pro Asp Asn Leu Lys Ala
245 250 255

Ile Gly Glu Leu Ala Phe Phe Asp Asn Gln Ile Thr Gly Lys Leu Ser
260 265 270

Leu Pro Arg Gln Leu Met Arg Leu Ala Glu Arg Ala Phe Lys Ser Asn
275 280 285

His Ile Lys Thr Ile Glu Phe Arg Gly Asn Ser Leu Lys Val Ile Gly
290 295 300

Glu Ala Ser Phe Gln Asp Asn Asp Leu Ser Gln Leu Met Leu Pro Asp
305 310 315 320

Gly Leu Glu Lys Ile Glu Ser Glu Ala Phe Thr Gly Asn Pro Gly Asp
325 330 335

Asp His Tyr Asn Asn Arg Val Val Leu Trp Thr Lys Ser Gly Lys Asn
340 345 350

Pro Tyr Gly Leu Ala Thr Glu Asn Thr Tyr Val Asn Pro Asp Lys Ser
355 360 365

Leu Trp Gln Glu Ser Pro Glu Ile Asp Tyr Thr Lys Trp Leu Glu Glu
370 375 380

Asp Phe Thr Tyr Gln Lys Asn Ser Val Thr Gly Phe Ser Asn Lys Gly
385 390 395 400

Leu Gln Lys Val Lys Arg Asn Lys Asn Leu Glu Ile Pro Lys Gln His
405 410 415

Asn Gly Val Thr Ile Thr Glu Ile Gly Asp Asn Ala Phe Arg Asn Val
420 425 430

Asp Phe Gln Asn Lys Thr Leu Arg Lys Tyr Asp Leu Glu Glu Val Lys
435 440 445

Leu Pro Ser Thr Ile Arg Lys Ile Gly Ala Phe Ala Phe Gln Ser Asn
450 455 460

Asn Leu Lys Ser Phe Glu Ala Ser Asp Asp Leu Glu Glu Ile Lys Glu
465 470 475 480

Gly Ala Phe Met Asn Asn Arg Ile Glu Thr Leu Glu Leu Lys Asp Lys
485 490 495

Leu Val Thr Ile Gly Asp Ala Ala Phe His Ile Asn His Ile Tyr Ala
500 505 510

Ile Val Leu Pro Glu Ser Val Gln Glu Ile Gly Arg Ser Ala Phe Arg
515 520 525

Gln Asn Gly Ala Asn Asn Leu Ile Phe Met Gly Ser Lys Val Lys Thr
530 535 540

Leu Gly Glu Met Ala Phe Leu Ser Asn Arg Leu Glu His Leu Asp Leu
545 550 555 560

Ser Glu Gln Lys Gln Leu Thr Glu Ile Pro Val Gln Ala Phe Ser Asp
565 570 575

Asn Ala Leu Lys Glu Val Leu Leu Pro Ala Ser Leu Lys Thr Ile Arg
580 585 590

Glu Glu Ala Phe Lys Lys Asn His Leu Lys Gln Leu Glu Val Ala Ser
595 600 605

Ala Leu Ser His Ile Ala Phe Asn Ala Leu Asp Asp Asn Asp Gly Asp
610 615 620

Glu Gln Phe Asp Asn Lys Val Val Val Lys Thr His His Asn Ser Tyr
625 630 635 640

Ala Leu Ala Asp Gly Glu His Phe Ile Val Asp Pro Asp Lys Leu Ser
645 650 655

Ser Thr Met Ile Asp Leu Glu Lys Ile Leu Lys Leu Ile Glu Gly Leu
660 665 670

Asp Tyr Ser Thr Leu Arg Gln Thr Thr Gln Thr Gln Phe Arg Asp Met
675 680 685

Thr Thr Ala Gly Lys Ala Leu Leu Ser Lys Ser Asn Leu Arg Gln Gly
690 695 700

Glu Lys Gln Lys Phe Leu Gln Glu Ala Gln Phe Phe Leu Gly Arg Val
705 710 715 720

Asp Leu Asp Lys Ala Ile Ala Lys Ala Glu Lys Ala Leu Val Thr Lys
725 730 735

Lys Ala Thr Lys Asn Gly Gln Leu Leu Glu Arg Ser Ile Asn Lys Ala
740 745 750

Val Leu Ala Tyr Asn Asn Ser Ala Ile Lys Lys Ala Asn Val Lys Arg
755 760 765

Leu Glu Lys Glu Leu Asp Leu Leu Thr Gly Leu Val Glu Gly Lys Gly
770 775 780

Pro Leu Ala Gln Ala Thr Met Val Gln Gly Val Tyr Leu Leu Lys Thr
785 790 795 800

Pro Leu Pro Leu Pro Glu Tyr Tyr Ile Gly Leu Asn Val Tyr Phe Asp
805 810 815

Lys Ser Gly Lys Leu Ile Tyr Ala Leu Asp Met Ser Asp Thr Ile Gly
820 825 830

Glu Gly Gln Lys Asp Ala Tyr Gly Asn Pro Ile Leu Asn Val Asp Glu
835 840 845

Asp Asn Glu Gly Tyr His Ala Leu Ala Val Ala Thr Leu Ala Asp Tyr
850 855 860

Glu Gly Leu Asp Ile Lys Thr Ile Leu Asn Ser Lys Leu Ser Gln Leu
865 870 875 880

Thr Ser Ile Arg Gln Val Pro Thr Ala Ala Tyr His Arg Ala Gly Ile
885 890 895

Phe Gln Ala Ile Gln Asn Ala Ala Ala Glu Ala Glu Gln Leu Leu Pro
900 905 910

Lys Pro Gly Met His Ser Glu Lys Ser Ser Ser Ser Glu Ser Ala Asn
915 920 925

Ser Lys Asp Arg Gly Leu Gln Ser His Pro Lys Thr Asn Arg Gly Arg
930 935 940

His Ser Ala Ile Leu Pro Arg Thr Gly Ser Lys Gly Ser Phe Val Tyr
945 950 955 960

Gly Ile Leu Gly Tyr Thr Ser Val Ala Leu Leu
965 970

<210> 7
<211> 971
<212> PRT
<213> Streptococcus pyogenes strain Spy60

<400> 7

Leu Val Lys Glu Pro Ile Leu Lys Gln Thr Gln Ala Ser Ser Ser Ile
1 5 10 15

Ser Gly Ala Asp Tyr Ala Glu Ser Ser Gly Lys Ser Lys Leu Lys Ile
20 25 30

Asn Glu Thr Ser Gly Pro Val Asp Asp Thr Val Thr Asp Leu Phe Ser
35 40 45

Asp Lys Arg Thr Thr Pro Glu Lys Ile Lys Asp Asn Leu Ala Lys Gly
50 55 60

Pro Arg Glu Gln Glu Leu Lys Ala Val Thr Glu Asn Thr Glu Ser Glu
65 70 75 80

Lys Gln Ile Thr Ser Gly Ser Gln Leu Glu Gln Ser Lys Glu Ser Leu
85 90 95

Ser Leu Asn Lys Thr Val Pro Ser Thr Ser Asn Trp Glu Ile Cys Asp
100 105 110

Phe Ile Thr Lys Gly Asn Thr Leu Val Gly Leu Ser Lys Ser Gly Val
115 120 125

Glu Lys Leu Ser Gln Thr Asp His Leu Val Leu Pro Ser Gln Ala Ala
130 135 140

Asp Gly Thr Gln Leu Ile Gln Val Ala Ser Phe Ala Phe Thr Pro Asp
145 150 155 160

Lys Lys Thr Ala Ile Ala Glu Tyr Thr Ser Arg Ala Gly Glu Asn Gly
165 170 175

Glu Ile Ser Gln Leu Asp Val Asp Gly Lys Glu Ile Ile Asn Glu Gly
180 185 190

Glu Val Phe Asn Ser Tyr Leu Leu Lys Lys Val Thr Ile Pro Thr Gly
195 200 205

Tyr Lys His Ile Gly Gln Asp Ala Phe Val Asp Asn Lys Asn Ile Ala
210 215 220

Glu Val Asn Leu Pro Glu Ser Leu Glu Thr Ile Ser Asp Tyr Ala Phe
225 230 235 240

Ala His Leu Ala Leu Lys Gln Ile Asp Leu Pro Asp Asn Leu Lys Ala
245 250 255

Ile Gly Glu Leu Ala Phe Phe Asp Asn Gln Ile Thr Gly Lys Leu Ser
260 265 270

Leu Pro Arg Gln Leu Met Arg Leu Ala Glu Arg Ala Phe Lys Ser Asn
275 280 285

His Ile Lys Thr Ile Glu Phe Arg Gly Asn Ser Leu Lys Val Ile Gly
290 295 300

Glu Ala Ser Phe Gln Asp Asn Asp Leu Ser Gln Leu Met Leu Pro Asp
305 310 315 320

Gly Leu Glu Lys Ile Glu Ser Glu Ala Phe Thr Gly Asn Pro Gly Asp
325 330 335

Asp His Tyr Asn Asn Arg Val Val Leu Trp Thr Lys Ser Gly Lys Asn
340 345 350

Pro Ser Gly Leu Ala Thr Glu Asn Thr Tyr Val Asn Pro Asp Lys Ser
355 360 365

Leu Trp Gln Glu Ser Pro Glu Ile Asp Tyr Thr Lys Trp Leu Glu Glu
370 375 380

Asp Phe Thr Tyr Gln Lys Asn Ser Val Thr Gly Phe Ser Asn Lys Gly
385 390 395 400

Leu Gln Lys Val Lys Arg Asn Lys Asn Leu Glu Ile Pro Lys Gln His
405 410 415

Asn Gly Val Thr Ile Thr Glu Ile Gly Asp Asn Ala Phe Arg Asn Val
420 425 430

Asp Phe Gln Asn Lys Thr Leu Arg Lys Tyr Asp Leu Glu Glu Val Lys
435 440 445

Leu Pro Ser Thr Ile Arg Lys Ile Gly Ala Phe Ala Phe Gln Ser Asn
450 455 460

Asn Leu Lys Ser Phe Glu Ala Ser Asp Asp Leu Glu Glu Ile Lys Glu
465 470 475 480

Gly Ala Phe Met Asn Asn Arg Ile Glu Thr Leu Glu Leu Lys Asp Lys
485 490 495

Leu Val Thr Ile Gly Asp Ala Ala Phe His Ile Asn His Ile Tyr Ala
500 505 510

Ile Val Leu Pro Glu Ser Val Gln Glu Ile Gly Arg Ser Ala Phe Arg
515 520 525

Gln Asn Gly Ala Asn Asn Leu Ile Phe Met Gly Ser Lys Val Lys Thr
530 535 540

Leu Gly Glu Met Ala Phe Leu Ser Asn Arg Leu Glu His Leu Asp Leu
545 550 555 560

Ser Glu Gln Lys Gln Leu Thr Glu Ile Pro Val Gln Ala Phe Ser Asp
565 570 575

Asn Ala Leu Lys Glu Val Leu Leu Pro Ala Ser Leu Lys Thr Ile Arg
580 585 590

Glu Glu Ala Phe Lys Lys Asn His Leu Lys Gln Leu Glu Val Ala Ser
595 600 605

Ala Leu Ser His Ile Ala Phe Asn Ala Leu Asp Asp Asn Asp Gly Asp
610 615 620

Glu Gln Phe Asp Asn Lys Val Val Val Lys Thr His His Asn Ser Tyr
625 630 635 640

Ala Leu Ala Asp Gly Glu His Phe Ile Val Asp Pro Asp Lys Leu Ser
645 650 655

Ser Thr Ile Val Asp Leu Glu Lys Ile Leu Lys Leu Ile Glu Gly Leu
660 665 670

Asp Tyr Ser Thr Leu Arg Gln Thr Thr Gln Thr Gln Phe Arg Asp Met
675 680 685

Thr Thr Ala Gly Lys Ala Leu Leu Ser Lys Ser Asn Leu Arg Gln Gly
690 695 700

Glu Lys Gln Lys Phe Leu Gln Glu Ala Gln Phe Phe Leu Gly Arg Val
705 710 715 720

Asp Leu Asp Lys Ala Ile Ala Lys Ala Glu Lys Ala Leu Val Thr Lys
725 730 735

Lys Ala Thr Lys Asn Gly Gln Leu Leu Glu Arg Ser Ile Asn Lys Ala
740 745 750

Val Leu Ala Tyr Asn Asn Ser Ala Ile Lys Lys Ala Asn Val Lys Arg
755 760 765

Leu Glu Lys Glu Leu Asp Leu Leu Thr Gly Leu Val Glu Gly Lys Gly
770 775 780

Pro Leu Ala Gln Ala Thr Met Val Gln Gly Val Tyr Leu Leu Lys Thr
785 790 795 800

Pro Leu Pro Leu Pro Glu Tyr Tyr Ile Gly Leu Asn Val Tyr Phe Asp
805 810 815

Lys Ser Gly Lys Leu Ile Tyr Ala Leu Asp Met Ser Asp Thr Ile Gly
820 825 830

Glu Gly Gln Lys Asp Ala Tyr Gly Asn Pro Ile Leu Asn Val Asp Glu
835 840 845

Asp Asn Glu Gly Tyr His Ala Leu Ala Val Ala Thr Leu Ala Asp Tyr
850 855 860

Glu Gly Leu Asp Ile Lys Thr Ile Leu Asn Ser Lys Leu Ser Gln Leu
865 870 875 880

Thr Ser Ile Arg Gln Val Pro Thr Ala Ala Tyr His Arg Ala Gly Ile
885 890 895

Phe Gln Ala Ile Gln Asn Ala Ala Ala Glu Ala Glu Gln Leu Leu Pro
900 905 910

Lys Pro Gly Thr His Ser Glu Lys Ser Ser Ser Ser Glu Ser Ala Asn
915 920 925

Ser Lys Asp Arg Gly Leu Gln Ser Asn Pro Lys Thr Asn Arg Gly Arg
930 935 940

His Ser Ala Ile Leu Pro Arg Thr Gly Ser Lys Gly Ser Phe Val Tyr
945 950 955 960

Gly Ile Leu Gly Tyr Thr Ser Val Ala Leu Leu
965 970

<210> 8
<211> 969
<212> PRT
<213> Streptococcus pyogenes strain ATCC12357

<400> 8
Val Lys Glu Pro Ile Leu Lys Gln Thr Gln Ala Ser Ser Ser Ile Ser
1 5 10 15

Gly Ala Asp Tyr Ala Glu Ser Ser Gly Lys Ser Lys Leu Lys Ile Asn
20 25 30

Glu Thr Ser Gly Pro Val Asp Asp Thr Val Thr Asp Leu Phe Ser Asp
35 40 45

Lys Arg Thr Thr Pro Glu Lys Ile Lys Asp Asn Leu Ala Lys Gly Pro
50 55 60

Arg Glu Gln Glu Leu Lys Ala Val Thr Glu Asn Thr Glu Ser Glu Lys
65 70 75 80

Gln Ile Asn Ser Gly Ser Gln Leu Glu Gln Ser Lys Glu Ser Leu Ser
85 90 95

Leu Asn Lys Arg Val Pro Ser Thr Ser Asn Trp Glu Ile Cys Asp Phe
100 105 110

Ile Thr Lys Gly Asn Thr Leu Val Gly Leu Ser Lys Ser Gly Val Glu
115 120 125

Lys Leu Ser Gln Thr Asp His Leu Val Leu Pro Ser Gln Ala Ala Asp
130 135 140

Gly Thr Gln Leu Ile Gln Val Ala Ser Phe Ala Phe Thr Pro Asp Lys
145 150 155 160

Lys Thr Ala Ile Ala Glu Tyr Thr Ser Arg Ala Gly Glu Asn Gly Glu
165 170 175

Ile Ser Gln Leu Asp Val Asp Gly Lys Glu Ile Ile Asn Glu Gly Glu
180 185 190

Val Phe Asn Ser Tyr Leu Leu Lys Lys Val Thr Ile Pro Thr Gly Tyr
195 200 205

Lys His Ile Gly Gln Asp Ala Phe Val Asp Asn Lys Asn Ile Ala Glu
210 215 220

Val Asn Leu Pro Glu Ser Leu Glu Thr Ile Ser Asp Tyr Ala Phe Ala
225 230 235 240

His Leu Ala Leu Lys Gln Ile Asp Leu Pro Asp Asn Leu Lys Ala Ile
245 250 255

Gly Glu Leu Ala Phe Phe Asp Asn Gln Ile Thr Gly Lys Leu Ser Leu
260 265 270

Pro Arg Gln Leu Met Arg Leu Ala Glu Arg Ala Phe Lys Ser Asn His
275 280 285

Ile Lys Thr Ile Glu Phe Arg Gly Asn Ser Leu Lys Val Ile Gly Glu
290 295 300

Ala Ser Phe Gln Asp Asn Asp Leu Ser Gln Leu Met Leu Pro Asp Gly
305 310 315 320

Leu Glu Lys Ile Glu Ser Glu Ala Phe Thr Gly Asn Pro Gly Asp Asp
325 330 335

His Tyr Asn Asn Arg Val Val Leu Trp Thr Lys Ser Gly Lys Asn Pro
340 345 350

Tyr Gly Leu Ala Thr Glu Asn Thr Tyr Val Asn Pro Asp Lys Ser Leu
355 360 365

Trp Gln Glu Ser Pro Glu Ile Asp Tyr Thr Lys Trp Leu Glu Glu Asp
370 375 380

Phe Thr Tyr Gln Lys Asn Ser Val Thr Gly Phe Ser Ser Lys Gly Leu
385 390 395 400

Gln Lys Val Lys Arg Asn Lys Asn Leu Glu Ile Pro Lys Gln His Asn
405 410 415

Gly Val Thr Ile Thr Glu Ile Gly Asp Asn Ala Phe Arg Asn Val Asp
420 425 430

Phe Gln Asn Lys Thr Leu Arg Lys Tyr Asp Leu Glu Glu Val Lys Leu
435 440 445

Pro Ser Thr Ile Arg Lys Ile Gly Ala Phe Ala Phe Gln Ser Asn Asn
450 455 460

Leu Lys Ser Phe Glu Ala Ser Asp Asp Leu Glu Glu Ile Lys Glu Gly
465 470 475 480

Ala Phe Met Asn Asn Arg Ile Glu Thr Leu Glu Leu Lys Asp Lys Leu
485 490 495

Val Thr Ile Gly Asp Ala Ala Phe His Ile Asn His Ile Tyr Ala Ile
500 505 510

Val Leu Pro Glu Ser Val Gln Glu Ile Gly Arg Ser Ala Phe Arg Gln
515 520 525

Asn Gly Ala Asn Asn Leu Ile Phe Met Gly Ser Lys Val Lys Thr Leu
530 535 540

Gly Glu Met Ala Phe Leu Ser Asn Arg Leu Glu His Leu Asp Leu Ser
545 550 555 560

Glu Gln Lys Gln Leu Thr Glu Ile Pro Val Gln Ala Phe Ser Asp Asn
565 570 575

Ala Leu Lys Glu Val Leu Leu Pro Ala Ser Leu Lys Thr Ile Arg Glu
580 585 590

Glu Ala Phe Lys Lys Asn His Leu Lys Gln Leu Glu Val Ala Ser Ala
595 600 605

Leu Ser His Ile Ala Phe Asn Ala Leu Asp Asp Asn Asp Gly Asp Glu
610 615 620

Gln Phe Asp Asn Lys Val Val Val Lys Thr His His Asn Ser Tyr Ala
625 630 635 640

Leu Ala Asp Gly Glu His Phe Ile Val Asp Pro Asp Lys Leu Ser Ser
645 650 655

Thr Ile Val Asp Leu Glu Lys Ile Leu Lys Leu Ile Glu Gly Leu Asp
660 665 670

Tyr Ser Thr Leu Arg Gln Thr Thr Gln Thr Gln Phe Arg Asp Met Thr
675 680 685

Thr Ala Gly Lys Ala Leu Leu Ser Lys Ser Asn Leu Arg Gln Gly Glu
690 695 700

Lys Gln Lys Phe Leu Gln Glu Ala Gln Phe Phe Leu Gly Arg Val Asp
705 710 715 720

Leu Asp Lys Ala Ile Ala Lys Ala Glu Lys Ala Leu Val Thr Lys Lys
725 730 735

Ala Thr Lys Asn Gly Gln Leu Leu Glu Arg Ser Ile Asn Lys Ala Val
740 745 750

Leu Ala Tyr Asn Asn Ser Ala Ile Lys Lys Ala Asn Val Lys Arg Leu
755 760 765

Glu Lys Glu Leu Asp Leu Leu Thr Gly Leu Val Glu Gly Lys Gly Pro
770 775 780

Leu Ala Gln Ala Thr Met Val Gln Gly Val Tyr Leu Leu Lys Thr Pro
785 790 795 800

Leu Pro Leu Pro Glu Tyr Tyr Ile Gly Leu Asn Val Tyr Phe Asp Lys
805 810 815

Ser Gly Lys Leu Ile Tyr Ala Leu Asp Met Ser Asp Thr Ile Gly Glu
820 825 830

Gly Gln Lys Asp Ala Tyr Gly Asn Pro Ile Leu Asn Val Asp Glu Asp
835 840 845

Asn Glu Gly Tyr His Ala Leu Ala Val Ala Thr Leu Ala Asp Tyr Glu
850 855 860

Gly Leu Asp Ile Lys Thr Ile Leu Asn Ser Lys Leu Ser Gln Leu Thr
865 870 875 880

Ser Ile Arg Gln Val Pro Thr Ala Ala Tyr His Arg Ala Gly Ile Phe
885 890 895

Gln Ala Ile Gln Asn Ala Ala Ala Glu Ala Glu Gln Leu Leu Pro Lys
900 905 910

Pro Gly Thr His Ser Glu Lys Ser Ser Ser Ser Glu Ser Ala Asn Ser
915 920 925

Lys Asp Arg Gly Leu Gln Ser Asn Pro Lys Thr Asn Arg Gly Arg His
930 935 940

Ser Ala Ile Leu Pro Arg Thr Gly Ser Lys Gly Ser Phe Val Tyr Gly
945 950 955 960

Ile Leu Gly Tyr Thr Ser Val Ala Leu
965

<210> 9
<211> 29
<212> DNA
<213> Artificial/Unknown

<220>
<221> misc_feature
<222> (1)..(29)
<223> primer

<400> 9
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<223> primer

<400> 12
ggttgtcgac agtaaagcaa cgctagtg 28

CLAIMS:

1. An isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide comprising SEQ ID NO: 2;

(b) a polypeptide comprising an antigenic or immunogenic fragment having at least 10 contiguous amino acid residues of the polypeptide of (a);

(c) a polypeptide comprising an antigenic or immunogenic analog having at least 70% identity to the polypeptide of (a) or (b);

(d) a polypeptide comprising an antigenic or immunogenic analog having at least 95% identity to the polypeptide of (a) or (b);

(e) a polypeptide capable of generating antibodies having binding specificity for the polypeptide of any one of (a), (b), (c) and (d);

(f) an epitope bearing portion of the polypeptide of any one of (a), (b), (c) and (d);

(g) the polypeptide of any one of (a), (b), (c), (d), (e) and (f) wherein the N-terminal Met residue is deleted; and

(h) the polypeptide of any one of (a), (b), (c), (d), (e), (f) and (g) wherein the secretory amino acid sequence is deleted.

2. An isolated polypeptide comprising a polypeptide chosen from:

(a) a polypeptide comprising SEQ ID NO: 2;

(b) a polypeptide having at least 70% identity to the polypeptide of (a);

(c) a polypeptide having at least 95% identity to the polypeptide of (a);

(d) a polypeptide capable of generating antibodies having binding specificity for the polypeptide of (a);

(e) an epitope bearing portion of the polypeptide of (a);

(f) the polypeptide of any one of (a), (b), (c), (d) and (e) wherein the N-terminal Met residue is deleted; and

(g) the polypeptide of any one of (a), (b), (c), (d), (e) and (f) wherein the secretory amino acid sequence is deleted.

3. A chimeric polypeptide comprising two or more of the polypeptide according to claim 1 or claim 2, provided that the polypeptides are linked so as to form a chimeric polypeptide.

4. An isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide comprising SEQ ID NO: 1;

(b) a polynucleotide encoding the polypeptide of claim 1; and

(c) a polynucleotide that is complementary to the polynucleotide in (a) or (b).

5. An isolated polynucleotide comprising a polynucleotide chosen from:

(a) a polynucleotide comprising SEQ ID NO: 1;

(b) a polynucleotide encoding the polypeptide of claim 2; and

(c) a polynucleotide that is complementary to the polynucleotide in (a) or (b).

6. The polynucleotide of claim 4 or claim 5, wherein said polynucleotide is DNA.

7. The polynucleotide of claim 4 or claim 5, wherein said polynucleotide is RNA.

8. The polynucleotide of claim 4 that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises SEQ ID NO: 2, or an antigenic or immunogenic fragment or an antigenic or immunogenic analog thereof.

9. The polynucleotide of claim 5 that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises SEQ ID NO: 2.

10. The polynucleotide of claim 4 that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising SEQ ID NO: 2, or an antigenic or immunogenic fragment or an antigenic or immunogenic analog thereof.

11. The polynucleotide of claim 5 that hybridizes under stringent conditions to either

(a) a DNA sequence encoding a polypeptide or

(b) the complement of a DNA sequence encoding a polypeptide;

wherein said polypeptide comprises at least 10 contiguous amino acid residues from a polypeptide comprising SEQ ID NO: 2.

12. A vector comprising the polynucleotide of claim 4 or claim 5, wherein said DNA is operably linked to an expression control region.

13. A host cell transfected with the vector of claim 12.

14. A process for producing a polypeptide comprising culturing a host cell according to claim 13 under conditions suitable for expression of said polypeptide.

15. A pharmaceutical composition comprising the polypeptide according to claim 1 or claim 2 or the chimeric

polypeptide according to claim 3 and a pharmaceutically acceptable carrier, diluent or adjuvant.

16. A method for prophylactic or therapeutic treatment of pharyngitis, erysipelas and impetigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis in a host susceptible to pharyngitis, erysipelas and impetigo, scarlet fever, and invasive diseases such as bacteremia and necrotizing fasciitis and also toxic shock comprising administering to said host a prophylactic or therapeutic amount of a composition according to claim 15.

17. A method for prophylactic or therapeutic treatment of Streptococcus pyogenes bacterial infection in a host susceptible to Streptococcus pyogenes infection comprising administering to said host a prophylactic or therapeutic amount of a composition according to claim 15.

18. A method according to claim 16 or claim 17 wherein the host is an animal.

19. A method for diagnosis of streptococcal infection in a host susceptible to streptococcal infection comprising

(a) obtaining a biological sample from the host;

(b) incubating an antibody or functional fragment thereof reactive with a polypeptide of any one of claims 1 to 3 with the biological sample to form a mixture; and

(c) detecting specifically bound antibody or bound functional fragment in the mixture which indicates the presence of streptococcal infection.

20. A method for detection of antibody specific to Streptococcus antigen in a biological sample comprising

(a) obtaining a biological sample from a host;

(b) incubating one or more polypeptides of any one of claims 1 to 3 with the biological sample to form a mixture; and

(c) detecting specifically bound antigen in the mixture which indicates the presence of antibody specific to Streptococcus.

21. Use of the polypeptide according to any one of claims 1 to 3 in the manufacture of a medicament for the prophylactic or therapeutic treatment of streptococcal infection.

22. Use of the polypeptide according to any one of claims 1 to 3 for the prophylactic or therapeutic treatment of streptococcal infection.

23. Kit comprising a polypeptide according to any one of claims 1 to 3 for detection or diagnosis of streptococcal infection.

SMART & BIGGAR
OTTAWA, CANADA

PATENT AGENTS

Figure 1 (SEQ ID NO:1)

```
   1 ATGAAGAAAC ATCTTAAAAC AGTTGCCTTG ACCCTCACTA CAGTATCGGT AGTCACCCAC
  61 AATCAGGAAG TTTTTAGTTT AGTCAAAGAG CCAATTCTTA AACAAACTCA AGCTTCTTCA
 121 TCGATTTCTG GCGCTGACTA CGCAGAAAGT AGCGGTAAAA GCAAGTTAAA GATTAATGAA
 181 ACTTCTGGCC CTGTTGATGA TACAGTCACT GACTTATTTT CGGATAAACG TACTACTCCT
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 301 GAGAATACAG AATCAGAAAA GCAGATCACT TCTGGATCTC AACTAGAACA ATCAAAAGAG
 361 TCTCTTTCTT TAAATAAAAC AGTGCCATCA ACGTCTAATT GGGAGATTTG TGATTTTATT
 421 ACTAAGGGGA ATACCCTTGT TGGTCTTTCA AAATCAGGTG TTGAAAAGTT ATCTCAAACT
 481 GATCATCTCG TATTGCCTAG TCAAGCAGCA GATGGAACTC AATTGATACA AGTAGCTAGT
 541 TTTGCTTTTA CTCCAGATAA AAAGACGGCA ATTGCAGAAT ATACCAGTAG GGCTGGAGAA
 601 AATGGGGAAA TAAGCCAACT AGATGTGGAT GGAAAAGAAA TTATTAACGA AGGTGAGGTT
 661 TTTAATTCTT ATCTACTAAA GAAGGTAACA ATCCCAACTG GTTATAAACA TATTGGTCAA
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 781 ATTTCTGACT ATGCTTTTGC TCACCTAGCT TTGAAACAGA TCGATTTGCC AGATAATTTA
 841 AAAGCGATTG GAGAATTAGC TTTTTTTGAT AATCAAATTA CAGGTAAACT TTCTTTGCCA
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 961 TTTAGAGGAA ATAGTCTAAA AGTGATAGGG GAAGCTAGTT TTCAAGATAA TGATCTGAGT
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1081 GGAGATGATC ACTACAATAA CCGTGTTGTT TTGTGGACAA AATCTGGAAA AAATCCTTCT
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1261 GGTTTTTCAA ATAAAGGCTT ACAAAAAGTA AAACGTAATA AAAACTTAGA AATTCCAAAA
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1381 CAAAATAAAA CTTTACGTAA ATATGATTTG GAAGAAGTAA AGCTTCCCTC AACTATTCGG
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1621 CTTCCAGAAT CTGTACAAGA AATAGGGCGT TCAGCATTTC GGCAAAATGG TGCAAATAAT
1681 CTTATTTTTA TGGGAAGTAA GGTTAAGACC TTAGGTGAGA TGGCATTTTT ATCAAATAGA
1741 CTTGAACATC TGGATCTTTC TGAGCAAAAA CAGTTAACAG AGATTCCTGT TCAAGCCTTT
1801 TCAGACAATG CCTTGAAAGA AGTATTATTA CCAGCATCAC TGAAAACGAT TCGAGAAGAA
1861 GCCTTCAAAA AGAATCATTT AAAACAACTG GAAGTGGCAT CTGCCTTGTC CCATATTGCT
1921 TTTAATGCTT TAGATGATAA TGATGGTGAT GAACAATTTG ATAATAAAGT GGTTGTTAAA
1981 ACGCATCATA ATTCCTACGC ACTAGCAGAT GGTGAGCATT TTATCGTTGA TCCAGATAAG
2041 TTATCTTCTA CAATAGTAGA CCTTGAAAAG ATTTTAAAAC TAATCGAAGG TTTAGATTAT
2101 TCTACATTAC GTCAGACTAC TCAAACTCAG TTTAGAGACA TGACTACTGC AGGTAAAGCG
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2221 TTTTTCCTTG GCCGCGTTGA TTTGGATAAA GCCATAGCTA AAGCTGAGAA GGCTTTAGTG
2281 ACCAAGAAGG CAACAAAGAA TGGTCAGTTG CTTGAAAGAA GTATTAACAA AGCGGTATTA
2341 GCTTATAATA ATAGCGCTAT TAAAAAAGCT AATGTTAAGC GCTTGGAAAA AGAGTTAGAC
2401 TTGCTAACAG GATTAGTTGA GGGAAAAGGA CCATTAGCGC AAGCTACAAT GGTACAAGGA
2461 GTTTATTTAT TAAAGACGCC TTTGCCATTG CCAGAATATT ATATCGGATT GAACGTTTAT
2521 TTTGACAAGT CTGGAAAATT GATTTATGCA CTTGATATGA GTGATACTAT TGGCGAGGGA
2581 CAAAAAGACG CTTATGGTAA TCCTATATTA AATGTTGACG AGGATAATGA AGGTTATCAT
2641 GCCTTGGCAG TTGCCACTTT AGCTGATTAT GAGGGGCTCG ACATCAAAAC AATTTTAAAT
2701 AGTAAGCTTA GTCAATTAAC ATCTATTCGT CAGGTACCGA CTGCAGCCTA TCATAGAGCC
2761 GGTATTTTCC AAGCTATCCA AAATGCAGCG GCAGAAGCAG AGCAGTTATT GCCTAAACCA
2821 GGTACGCACT CTGAGAAGTC AAGCTCAAGT GAATCTGCTA ACTCTAAAGA TAGAGGATTG
2881 CAATCAAACC CAAAAACGAA TAGAGGACGA CACTCTGCAA TATTGCCTAG GACAGGGTCA
2941 AAAGGCAGCT TTGTCTATGG AATCTTAGGT TACACTAGCG TTGCTTTACT GTCACTAATA
3001 ACTGCTATAA AAAAGAAAAA ATATTAA
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Figure 2 (SEQ ID NO:2)

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   1 MKKHLKTVAL TLTTVSVVTH NQEVFSLVKE PILKQTQASS SISGADYAES SGKSKLKINE
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 121 SLSLNKTVPS TSNWEICDFI TKGNTLVGLS KSGVEKLSQT DHLVLPSQAA DGTQLIQVAS
 181 FAFTPDKKTA IAEYTSRAGE NGEISQLDVD GKEIINEGEV FNSYLLKKVT IPTGYKHIGQ
 241 DAFVDNKNIA EVNLPESLET ISDYAFAHLA LKQIDLPDNL KAIGELAFFD NQITGKLSLP
 301 RQLMRLAERA FKSNHIKTIE FRGNSLKVIG EASFQDNDLS QLMLPDGLEK IESEAFTGNP
 361 GDDHYNNRVV LWTKSGKNPS GLATENTYVN PDKSLWQESP EIDYTKWLEE DFTYQKNSVT
 421 GFSNKGLQKV KRNKNLEIPK QHNGVTITEI GDNAFRNVDF QNKTLRKYDL EEVKLPSTIR
 481 KIGAFAFQSN NLKSFEASDD LEEIKEGAFM NNRIETLELK DKLVTIGDAA FHINHIYAIV
 541 LPESVQEIGR SAFRQNGANN LIFMGSKVKT LGEMAFLSNR LEHLDLSEQK QLTEIPVQAF
 601 SDNALKEVLL PASLKTIREE AFKKNHLKQL EVASALSHIA FNALDDNDGD EQFDNKVVVK
 661 THHNSYALAD GEHFIVDPDK LSSTIVDLEK ILKLIEGLDY STLRQTTQTQ FRDMTTAGKA
 721 LLSKSNLRQG EKQKFLQEAQ FFLGRVDLDK AIAKAEKALV TKKATKNGQL LERSINKAVL
 781 AYNNSAIKKA NVKRLEKELD LLTGLVEGKG PLAQATMVQG VYLLKTPLPL PEYYIGLNVY
 841 FDKSGKLIYA LDMSDTIGEG QKDAYGNPIL NVDEDNEGYH ALAVATLADY EGLDIKTILN
 901 SKLSQLTSIR QVPTAAYHRA GIFQAIQNAA AEAEQLLPKP GTHSEKSSSS ESANSKDRGL
 961 QSNPKTNRGR HSAILPRTGS KGSFVYGILG YTSVALLSLI TAIKKKKY*
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Clustal W(1.4) multiple sequence alignment

7 Sequences Aligned.          Alignment Score = 118839
Gaps Inserted = 0             Conserved Identities = 936

Pairwise Alignment Mode: Fast
Pairwise Alignment Parameters:
    ktup = 1   Gap Penalty = 3   Top Diagonals = 5   Window Size = 5

Multiple Alignment Parameters:
    Open Gap Penalty = 10.0   Extend Gap Penalty = 0.1
    Delay Divergent = 40%     Gap Distance = 8
    Similarity Matrix: blosum

Processing time: 12.9 seconds


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Spy69_M6       1                                      KQTQASSSISGADYAES   17
Spy68_M2       1                                LVKEPILKQTQASSSISGADYAES   24
Spy60_M1       1                                LVKEPILKQTQASSSISGADYAES   24
12357_M18      1                                 VKEPILKQTQASSSISGADYAES   23
700294_M1      1 MKKHLKTVALTLTTVSVVTHNQEVFSLVKEPILKQTQASSSISGADYAES   50
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Spy74_M3       6 SGKSKLKINETSGPVDDTVTDLFSDKRTTPEKIKDNLAKGPREQELKAVT   55
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Spy69_M6      18 SGKSKLKINETSGPVDDTVTDLFSDKRTTPEKIKDNLAKGPREQELKAVT   67
Spy68_M2      25 SGKSKLKINETSGPVDDTVTDLFSDKRTTPEKIKDNLAKGPREQELKTVT   74
Spy60_M1      25 SGKSKLKINETSGPVDDTVTDLFSDKRTTPEKIKDNLAKGPREQELKAVT   74
12357_M18     24 SGKSKLKINETSGPVDDTVTDLFSDKRTTPEKIKDNLAKGPREQELKAVT   73
700294_M1     51 SGKSKLKINETSGPVDDTVTDLFSDKRTTPEKIKDNLAKGPREQELKAVT  100
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Spy70_M5      75 ENTESEKQINSGSQLEQSKESLSLNKRVPSTSNWEICDFITKGNTLVGLS  124
Spy69_M6      68 ENTESEKQINSGSQLEQSKESLSLNKRVPSTSNWEICDFITKGNTLVGLS  117
Spy68_M2      75 ENTESEKQITSGSQLEQSKESLSLNKTVPSTSNWEICDFITKGNTLVGLS  124
Spy60_M1      75 ENTESEKQITSGSQLEQSKESLSLNKTVPSTSNWEICDFITKGNTLVGLS  124
12357_M18     74 ENTESEKQINSGSQLEQSKESLSLNKRVPSTSNWEICDFITKGNTLVGLS  123
700294_M1    101 ENTESEKQITSGSQLEQSKESLSLNKTVPSTSNWEICDFITKGNTLVGLS  150
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Spy74_M3     106 KSGVEKLSQTDHLVLPSQAADGTQLIQVASFAFTPDKKTAIAEYTSRAGE  155
Spy70_M5     125 KSGVEKLSQTDHLVLPSQAADGTQLIQVASFAFTPDKKTAIAEYTSRAGE  174
Spy69_M6     118 KSGVEKLSQTDHLVLPSQAADGTQLIQVASFAFTPDKKTAIAEYTSRAGE  167
Spy68_M2     125 KSGVEKLSQTDHLVLPSQAADGTQLIQVASFAFTPDKKTAIAEYTSRAGE  174
Spy60_M1     125 KSGVEKLSQTDHLVLPSQAADGTQLIQVASFAFTPDKKTAIAEYTSRAGE  174
12357_M18    124 KSGVEKLSQTDHLVLPSQAADGTQLIQVASFAFTPDKKTAIAEYTSRAGE  173
700294_M1    151 KSGVEKLSQTDHLVLPSQAADGTQLIQVASFAFTPDKKTAIAEYTSRAGE  200
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FIG. 3

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Spy74_M3     156 NGEISQLDVDGKEIINEGEVFNSYLLKKVTIPTGYKHIGQDAFVDNKNIA  205
Spy70_M5     175 NGEISQLDVDGKEIINEGEVFNSYLLKKVTIPTGYKHIGQDAFVDNKNIA  224
Spy69_M6     168 NGEISQLDVDGKEIINEGEVFNSYLLKKVTIPTGYKHIGQDAFVDNKNIA  217
Spy68_M2     175 NGEISQLDVDGKEIINEGEVFNSYLLKKVTIPTGYKHIGQDAFVDNKNIA  224
Spy60_M1     175 NGEISQLDVDGKEIINEGEVFNSYLLKKVTIPTGYKHIGQDAFVDNKNIA  224
12357_M18    174 NGEISQLDVDGKEIINEGEVFNSYLLKKVTIPTGYKHIGQDAFVDNKNIA  223
700294_M1    201 NGEISQLDVDGKEIINEGEVFNSYLLKKVTIPTGYKHIGQDAFVDNKNIA  250
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Spy74_M3     206 EVNLPESLETISDYAFAHLALKQIDLPDNLKAIGELAFFDNQITGKLSLP  255
Spy70_M5     225 EVNLPESLETISDYAFAHLALKQIDLPDNLKAIGELAFFDNQITGKLSLP  274
Spy69_M6     218 EVNLPESLETISDYAFAHLALKQIDLPDNLKAIGELAFFDNQITGKLSLP  267
Spy68_M2     225 EVNLPESLETISDYAFAHLALKQIDLPDNLKAIGELAFFDNQITGKLSLP  274
Spy60_M1     225 EVNLPESLETISDYAFAHLALKQIDLPDNLKAIGELAFFDNQITGKLSLP  274
12357_M18    224 EVNLPESLETISDYAFAHLALKQIDLPDNLKAIGELAFFDNQITGKLSLP  273
700294_M1    251 EVNLPESLETISDYAFAHLALKQIDLPDNLKAIGELAFFDNQITGKLSLP  300
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Spy74_M3     256 RQLMRLAERAFKSNHIKTIEFRGNSLKVIGEASFQDNDLSQLMLPDGLEK  305
Spy70_M5     275 RQLMRLAERAFKSNHIKTIEFRGNSLKVIGEASFQDNDLSQLMLPDGLEK  324
Spy69_M6     268 RQLMRLAERAFKSNHIKTIEFRGNSLKVIGEASFQDNDLSQLMLPDGLEK  317
Spy68_M2     275 RQLMRLAERAFKSNHIKTIEFRGNSLKVIGEASFQDNDLSQLMLPDGLEK  324
Spy60_M1     275 RQLMRLAERAFKSNHIKTIEFRGNSLKVIGEASFQDNDLSQLMLPDGLEK  324
12357_M18    274 RQLMRLAERAFKSNHIKTIEFRGNSLKVIGEASFQDNDLSQLMLPDGLEK  323
700294_M1    301 RQLMRLAERAFKSNHIKTIEFRGNSLKVIGEASFQDNDLSQLMLPDGLEK  350
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Spy74_M3     306 IESEAFTGNPGDDHYNNRVVLWTKSGKNPYGLATENTYVNPDKSLWQESP  355
Spy70_M5     325 IESEAFTGNPGDDHYNNRVVLWTKSGKNPYGLATENTYVNPDKSLWQESP  374
Spy69_M6     318 IESEAFTGNPGDDHYNNRVVLWTKSGKNPYGLATENTYVNPDKSLWQESP  367
Spy68_M2     325 IESEAFTGNPGDDHYNNRVVLWTKSGKNPYGLATENTYVNPDKSLWQESP  374
Spy60_M1     325 IESEAFTGNPGDDHYNNRVVLWTKSGKNPSGLATENTYVNPDKSLWQESP  374
12357_M18    324 IESEAFTGNPGDDHYNNRVVLWTKSGKNPYGLATENTYVNPDKSLWQESP  373
700294_M1    351 IESEAFTGNPGDDHYNNRVVLWTKSGKNPSGLATENTYVNPDKSLWQESP  400
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Spy74_M3     356 EIDYTKWLEEDFTYQKNSVTGFSSKGLQKVKRNKNLEIPKQHNGVTITEI  405
Spy70_M5     375 EIDYTKWLEEDFTYQKNSVTGFSSKGLQKVKRNKNLEIPKQHNGVTITEI  424
Spy69_M6     368 EIDYTKWLEEDFTYQKNSVTGFSSKGLQKVKRNKNLEIPKQHNGVTITEI  417
Spy68_M2     375 EIDYTKWLEEDFTYQKNSVTGFSNKGLQKVKRNKNLEIPKQHNGVTITEI  424
Spy60_M1     375 EIDYTKWLEEDFTYQKNSVTGFSNKGLQKVKRNKNLEIPKQHNGVTITEI  424
12357_M18    374 EIDYTKWLEEDFTYQKNSVTGFSSKGLQKVKRNKNLEIPKQHNGVTITEI  423
700294_M1    401 EIDYTKWLEEDFTYQKNSVTGFSNKGLQKVKRNKNLEIPKQHNGVTITEI  450
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Spy74_M3     406 GDNAFRNVDFQNKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASDD  455
Spy70_M5     425 GDNAFRNVDFQNKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASDD  474
Spy69_M6     418 GDNAFRNVNFQNKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASDD  467
Spy68_M2     425 GDNAFRNVDFQNKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASDD  474
Spy60_M1     425 GDNAFRNVDFQNKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASDD  474
12357_M18    424 GDNAFRNVDFQNKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASDD  473
700294_M1    451 GDNAFRNVDFQNKTLRKYDLEEVKLPSTIRKIGAFAFQSNNLKSFEASDD  500
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FIG. 3
(continued)

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Spy74_M3     456 LEEIKEGAFMNNRIETLELKDKLVTIGDAAFHINHIYAIVLPESVQEIGR  505
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Spy69_M6     468 LEEIKEGAFMNNRIETLELKDKLVTIGDAAFHINHIYAIVLPESVQEIGR  517
Spy68_M2     475 LEEIKEGAFMNNRIETLELKDKLVTIGDAAFHINHIYAIVLPESVQEIGR  524
Spy60_M1     475 LEEIKEGAFMNNRIETLELKDKLVTIGDAAFHINHIYAIVLPESVQEIGR  524
12357_M18    474 LEEIKEGAFMNNRIETLELKDKLVTIGDAAFHINHIYAIVLPESVQEIGR  523
700294_M1    501 LEEIKEGAFMNNRIETLELKDKLVTIGDAAFHINHIYAIVLPESVQEIGR  550
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Spy74_M3     506 SAFRQNGANNLIFMGSKVKTIGEMAFLSNRLEHLDLSEQKQLTEIPVQAF  555
Spy70_M5     525 SAFRQNGANNLIFMGSKVKTLGEMAFLSNRLEHLDLSEQKQLTEIPVQAF  574
Spy69_M6     518 SAFRQNGANNLIFMGSKVKTLGEMAFLSNRLEHLDLSEQKQLTEIPVQAF  567
Spy68_M2     525 SAFRQNGANNLIFMGSKVKTLGEMAFLSNRLEHLDLSEQKQLTEIPVQAF  574
Spy60_M1     525 SAFRQNGANNLIFMGSKVKTLGEMAFLSNRLEHLDLSEQKQLTEIPVQAF  574
12357_M18    524 SAFRQNGANNLIFMGSKVKTLGEMAFLSNRLEHLDLSEQKQLTEIPVQAF  573
700294_M1    551 SAFRQNGANNLIFMGSKVKTLGEMAFLSNRLEHLDLSEQKQLTEIPVQAF  600
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Spy74_M3     556 SDNALKEVLLPASLKTIREEAFKKNHLKQLEVASALSHIAFNALDDNDGD  605
Spy70_M5     575 SDNALKEVLLPASLKTIREEAFKKNHLKQLEVASALSHIAFNALDDNDGD  624
Spy69_M6     568 SDNALKEVLLPASLKTIREEAFKKNHLKQLEVASALSHIAFNALDDNDGD  617
Spy68_M2     575 SDNALKEVLLPASLKTIREEAFKKNHLKQLEVASALSHIAFNALDDNDGD  624
Spy60_M1     575 SDNALKEVLLPASLKTIREEAFKKNHLKQLEVASALSHIAFNALDDNDGD  624
12357_M18    574 SDNALKEVLLPASLKTIREEAFKKNHLKQLEVASALSHIAFNALDDNDGD  623
700294_M1    601 SDNALKEVLLPASLKTIREEAFKKNHLKQLEVASALSHIAFNALDDNDGD  650
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Spy74_M3     606 EQFDNKVVVKTHHNSYALADGEHFIVDPDKLSSTMVDLEKILKLIEGLDY  655
Spy70_M5     625 EQFDNKVVVKTHHNSYALADGEHFIVDPDKLSSTIVDLEKILKLIEGLDY  674
Spy69_M6     618 EQFDNKVVVKTHHNSYALADGEHFIVDPDKLSSTIVDLEKILKLIEGLDY  667
Spy68_M2     625 EQFDNKVVVKTHHNSYALADGEHFIVDPDKLSSTMIDLEKILKLIEGLDY  674
Spy60_M1     625 EQFDNKVVVKTHHNSYALADGEHFIVDPDKLSSTIVDLEKILKLIEGLDY  674
12357_M18    624 EQFDNKVVVKTHHNSYALADGEHFIVDPDKLSSTIVDLEKILKLIEGLDY  673
700294_M1    651 EQFDNKVVVKTHHNSYALADGEHFIVDPDKLSSTIVDLEKILKLIEGLDY  700
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Spy74_M3     656 STLRQTTQTQFRDMTTAGKALLSKSKLRQGEKQKFLQEAQFFLGRVDLDK  705
Spy70_M5     675 STLRQTTQTQFRDMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGRVDLDK  724
Spy69_M6     668 STLRQTTQTQFRDMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGRVDLDK  717
Spy68_M2     675 STLRQTTQTQFRDMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGRVDLDK  724
Spy60_M1     675 STLRQTTQTQFRDMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGRVDLDK  724
12357_M18    674 STLRQTTQTQFRDMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGRVDLDK  723
700294_M1    701 STLRQTTQTQFRDMTTAGKALLSKSNLRQGEKQKFLQEAQFFLGRVDLDK  750
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Spy74_M3     706 AIAKAEKALVTKKATKNGQLLGRSINKAVLAYNNSAIKKANVKRLEKELD  755
Spy70_M5     725 AIAKAEKALVTKKATKNGQLLERSINKAVLAYNNSAIKKANVKRLEKELD  774
Spy69_M6     718 AIAKAEKALVTKKATKNGQLLERSINKAVSAYNNSAIKKANVKRLEKELD  767
Spy68_M2     725 AIAKAEKALVTKKATKNGQLLERSINKAVLAYNNSAIKKANVKRLEKELD  774
Spy60_M1     725 AIAKAEKALVTKKATKNGQLLERSINKAVLAYNNSAIKKANVKRLEKELD  774
12357_M18    724 AIAKAEKALVTKKATKNGQLLERSINKAVLAYNNSAIKKANVKRLEKELD  773
700294_M1    751 AIAKAEKALVTKKATKNGQLLERSINKAVLAYNNSAIKKANVKRLEKELD  800
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FIG. 3
(continued)

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Spy74_M3     756 LLTGLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA  805
Spy70_M5     775 LLTGLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA  824
Spy69_M6     768 LLTGLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA  817
Spy68_M2     775 LLTGLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA  824
Spy60_M1     775 LLTGLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA  824
12357_M18    774 LLTGLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA  823
700294_M1    801 LLTGLVEGKGPLAQATMVQGVYLLKTPLPLPEYYIGLNVYFDKSGKLIYA  850
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Spy74_M3     806 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLDIKTILN  855
Spy70_M5     825 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLDIKTILN  874
Spy69_M6     818 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLDIKTILN  867
Spy68_M2     825 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLDIKTILN  874
Spy60_M1     825 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLDIKTILN  874
12357_M18    824 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLDIKTILN  873
700294_M1    851 LDMSDTIGEGQKDAYGNPILNVDEDNEGYHALAVATLADYEGLDIKTILN  900
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Spy74_M3     856 SKLSQLTSIRQVPTAAYHRAGIFQAIQNAAAEAEQLLPKPGTHSEKSSSS  905
Spy70_M5     875 SKLSQLTSIRQVPTAAYHRAGIFQAIQNAAAEAEQLLPKAGTHSEKSSSS  924
Spy69_M6     868 SKLSQLTSIRQVPTAAYHRAGIFQAIQNAAAEAEQLLPKPGTHSEKSSSS  917
Spy68_M2     875 SKLSQLTSIRQVPTAAYHRAGIFQAIQNAAAEAEQLLPKPGMHSEKSSSS  924
Spy60_M1     875 SKLSQLTSIRQVPTAAYHRAGIFQAIQNAAAEAEQLLPKPGTHSEKSSSS  924
12357_M18    874 SKLSQLTSIRQVPTAAYHRAGIFQAIQNAAAEAEQLLPKPGTHSEKSSSS  923
700294_M1    901 SKLSQLTSIRQVPTAAYHRAGIFQAIQNAAAEAEQLLPKPGTHSEKSSSS  950
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Spy74_M3     906 ESANSKDRGLQSNPKTNRGRHSAILPRTGSKGSFVYGILGYTSVAL      951
Spy70_M5     925 ESANSKDRGLQSNPKTNRGRHSAILPRTGSKGSFVYGILGYTSVAL      970
Spy69_M6     918 ESANSKDRGLQSNPKTNRGRHSAILPRTGSKGSFVYGILGYTSVAL      963
Spy68_M2     925 ESANSKDRGLQSHPKTNRGRHSAILPRTGSKGSFVYGILGYTSVALL     971
Spy60_M1     925 ESANSKDRGLQSNPKTNRGRHSAILPRTGSKGSFVYGILGYTSVALL     971
12357_M18    924 ESANSKDRGLQSNPKTNRGRHSAILPRTGSKGSFVYGILGYTSVAL      969
700294_M1    951 ESANSKDRGLQSNPKTNRGRHSAILPRTGSKGSFVYGILGYTSVALLSLI 1000
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Spy74_M3     952           951 (SEQ ID NO:3)
Spy70_M5     971           970 (SEQ ID NO:4)
Spy69_M6     964           963 (SEQ ID NO:5)
Spy68_M2     972           971 (SEQ ID NO:6)
Spy60_M1     972           971 (SEQ ID NO:7)
12357_M18    970           969 (SEQ ID NO:8)
700294_M1   1001 TAIKKKKY 1008 (SEQ ID NO:2)
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FIG. 3
(continued)

AMENDED SHEET